THE MANGA GUIDE™ TO

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MOLECULAR BIOLOGY

MASAHARU TAKEMURA
SAKURA
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This English edition is co-published by No Starch Press and Ohmsha, Ltd.

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13 12 11 10 09 1 2 3 4 5 6 7 8 9

ISBN-10: 1-59327-202-2
ISBN-13: 978-1-59327-202-9

Publisher: William Pollock
Author: Masaharu Takemura
Illustrator: Sakura
Producer: Becom Co., Ltd.
Production Editors: Kathleen Mish and Magnolia Molcan
Developmental Editor: Tyler Ortman
Technical Reviewers: Read Siry, E. Jane Richardson, and Kerri Lendo
Compositor: Riley Hoffman
Proofreader: Cristina Chan
Indexer: Sarah Schott

For information on book distributors or translations, please contact No Starch Press, Inc. directly:
No Starch Press, Inc.
555 De Haro Street, Suite 250, San Francisco, CA 94107
phone: 415.863.9900; fax: 415.863.9950; info@nostarch.com; http://www.nostarch.com/

Library of Congress Cataloging-in-Publication Data

```
Takemura, Masaharu, 1969-
  [Manga de wakaru bunshi seibutsugaku. English]
  The manga guide to molecular biology / Masaharu Takemura, Sakura, Becom Co., Ltd.
       p. cm.
  Includes index.
  ISBN-13: 978-1-59327-202-9
  ISBN-10: 1-59327-202-2
 1.  Molecular biology--Comic books, strips, etc. 2.  Molecular biology--Popular works.  I. Becom Co.
II. Title.
  QH506.T3513 2009
  572.8--dc22
                                                                    2009025876
```

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PREFACE

Molecular biology is an academic discipline aimed at understanding the behavior of living organisms too small for our eyes to see. Genes play important roles in our world. However, they are not only invisible to our eyes but also difficult to observe even with a microscope.

Researchers of molecular biology such as myself are conducting many experiments every day in laboratories at colleges, research institutes, and corporations. Researchers work to understand the behavior of DNA, proteins, and RNA based on their experiments and to understand this small world using models they create.

Since we cannot see the subjects of our experiments, knowledge in the field of molecular biology tends to be based on experimental data—and there are still many things we do not understand. While this research itself is difficult to pursue, the more difficult task is conveying the world of molecular biology to nonspecialists in an easy-to-understand manner. *The Manga Guide to Molecular Biology* is an attempt to do just that.

The main characters of this book are two college girls, Ami Kasuga and Rin Natsukawa. These two girls are called to a small isolated island owned by Professor Moro for a molecular biology make-up class. Through a virtual reality machine that brings them into the world of molecular biology, they learn a lot, along with help from Marcus, the professor's handsome assistant.

Since the girls aim to grasp the big picture of molecular biology, this book contains many descriptions designed to facilitate readers' understanding of the subject. In other words, the processes of replication of DNA, gene transcription, and protein synthesis are not quite as simple as they seem in this book.

If readers come to feel that the world of molecular biology is more complex and contains many more areas they wish to understand, then more than half of the purpose of publishing this book, I think, will have been achieved.

Having said that, it also needs to be said that molecular biology is a profound academic discipline. It is inevitably linked to other areas of study, including medical science, agriculture, and engineering, as well as basic scientific areas, such as physics, chemistry, and geosciences, not to mention biology. And it is closely related to the daily lives of many people.

Thanks to research results that have increased at an explosive pace from the end of the 20th century and into this century, the field of molecular biology has vastly expanded. It is difficult for a single researcher now to have sufficient knowledge to grasp the entire picture of molecular biology.

This book covers only the basics of molecular biology. If you want to obtain a true picture of molecular biology, I recommend using this book as a beginning and then going on to nurture your interest in the variety of other materials available on this subject.

In conclusion, I would like to take this opportunity to thank all the staff at Ohmsha, Ltd., Mr. Masayoshi Maeda for the wonderful scenarios, Mr. Sakura for creating the amazing representation of the complex world of molecules in cartoon form, and, above all, the readers who have taken up this book.

MASAHARU TAKEMURA
JANUARY 2008

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FURTHER READING

In writing this book, the author referenced many academic papers and books written both in Japanese and English. Among these works, the following books are relatively easy to obtain in English.

Black, Jacquelyn G. *Microbiology: Principles and Explorations*. Hoboken: Wiley, 2008.

'Espinasse, Margaret. *Robert Hooke*. Berkeley: University of California Press, 1962.

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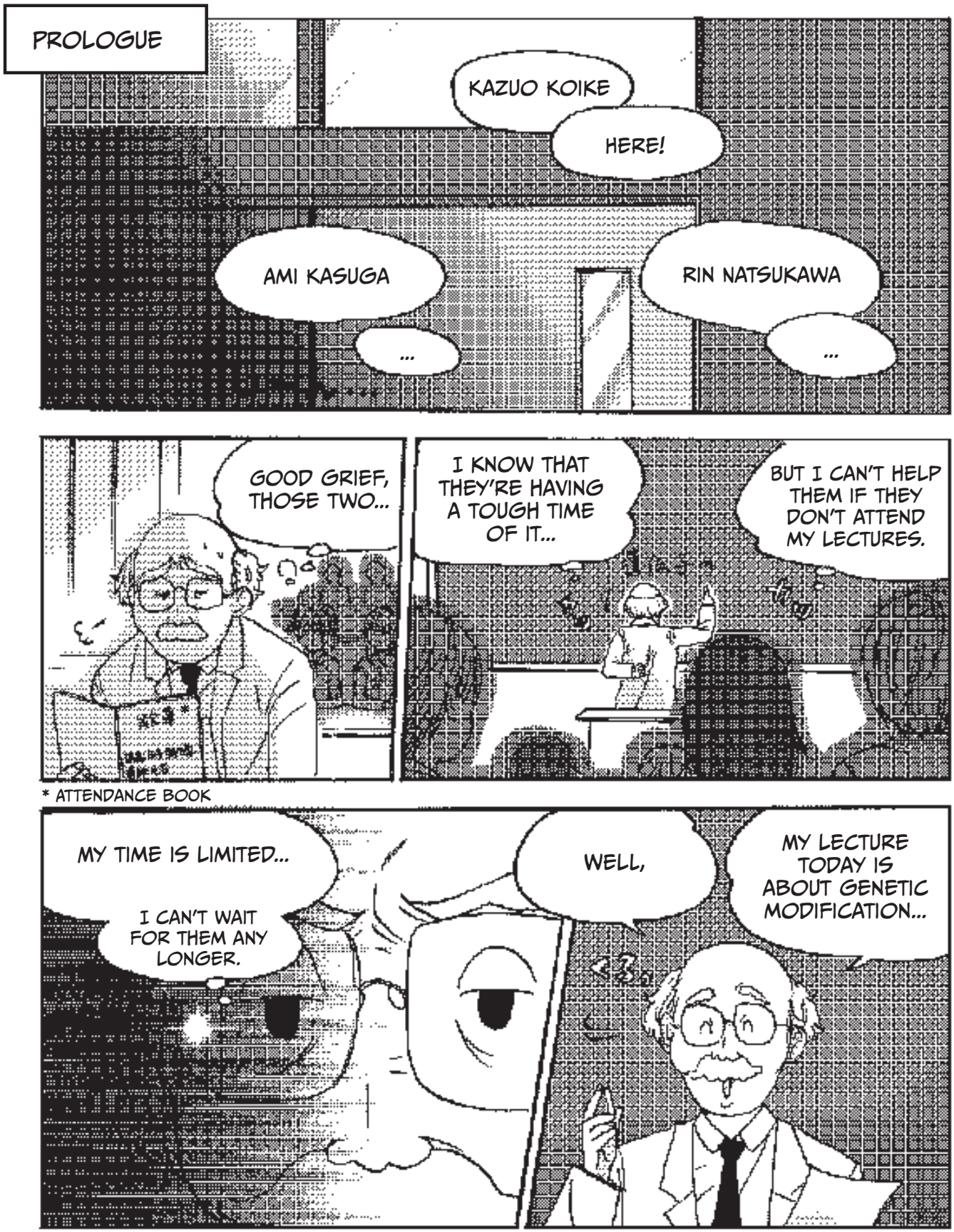

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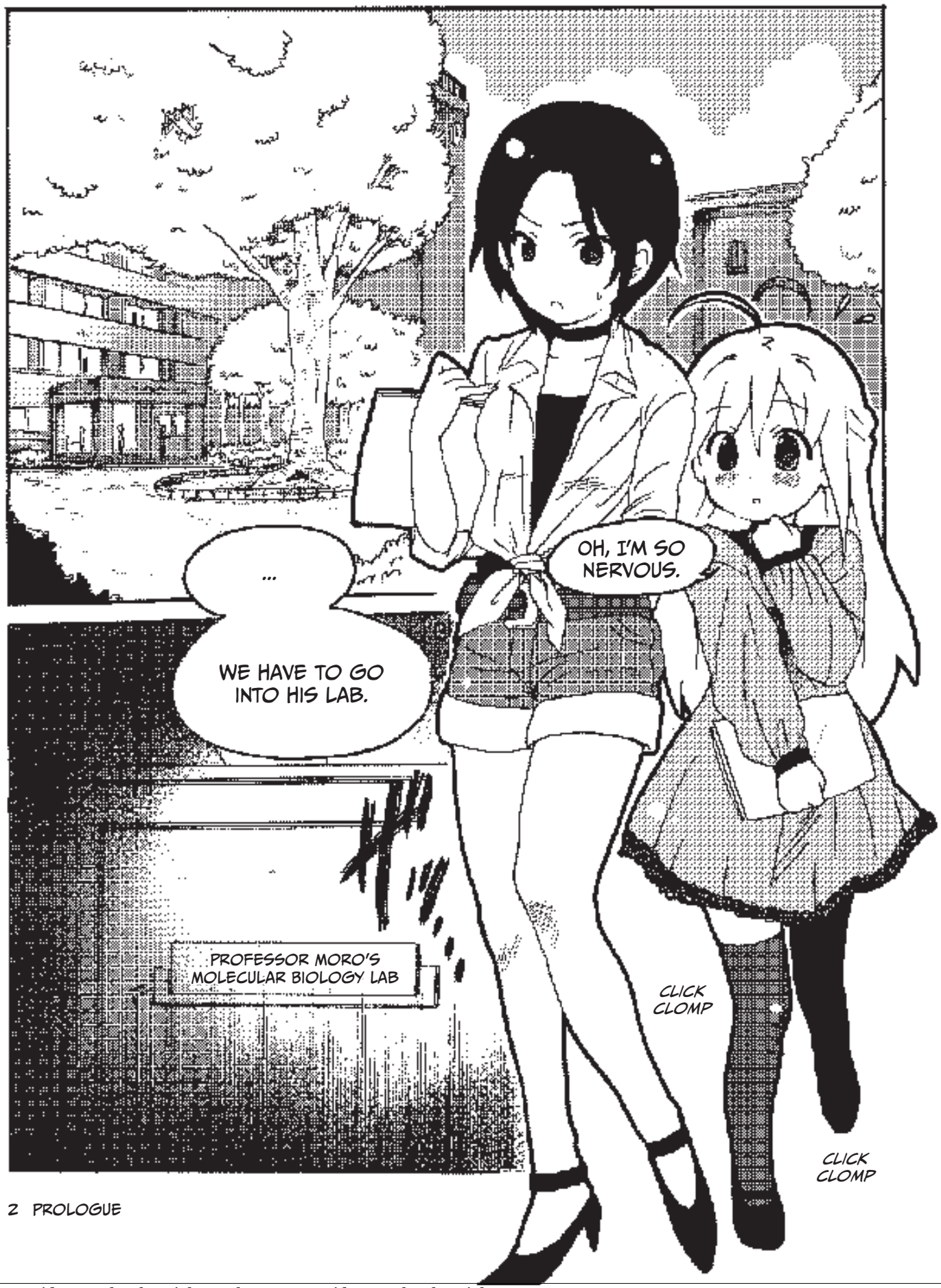

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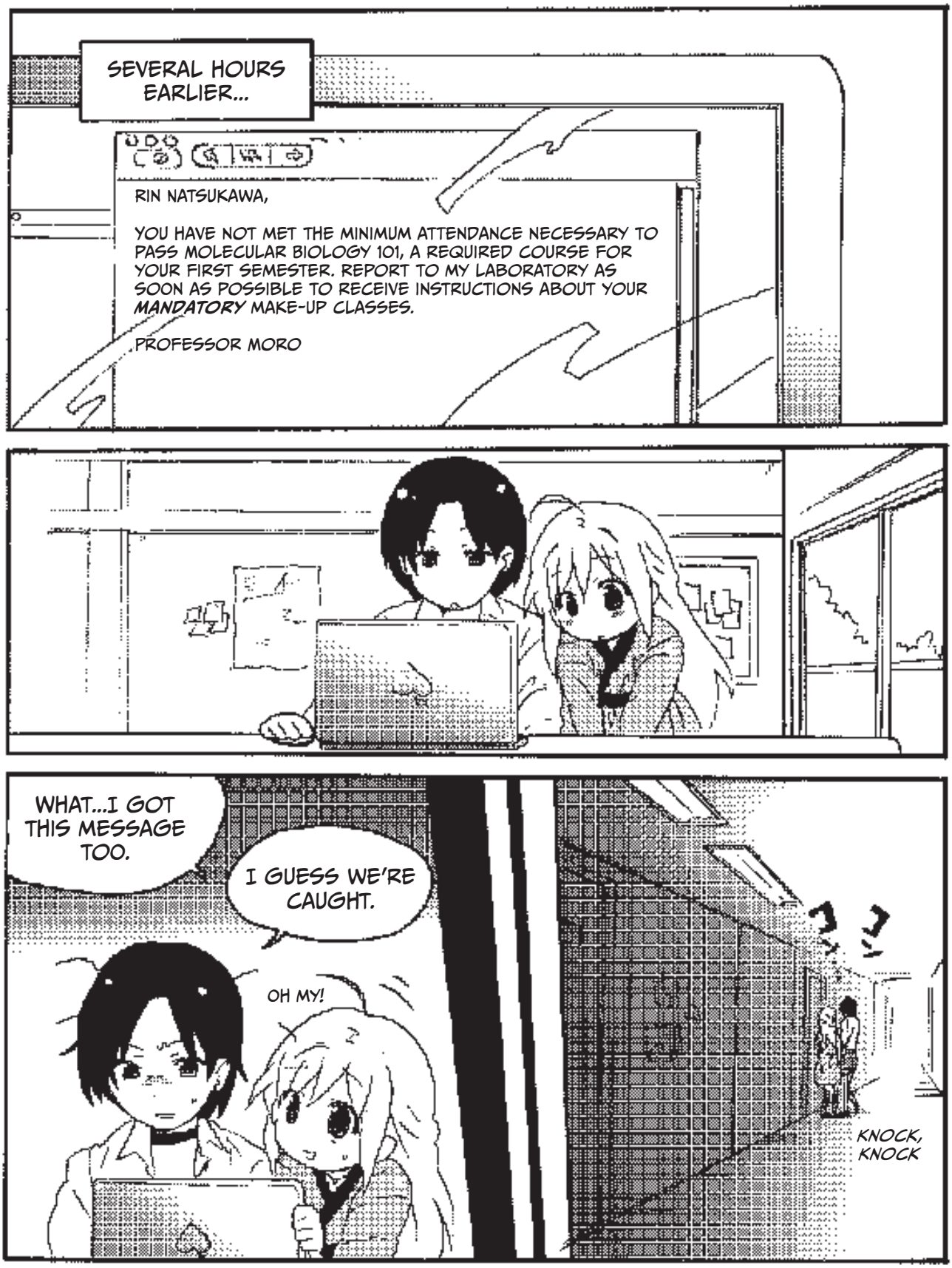

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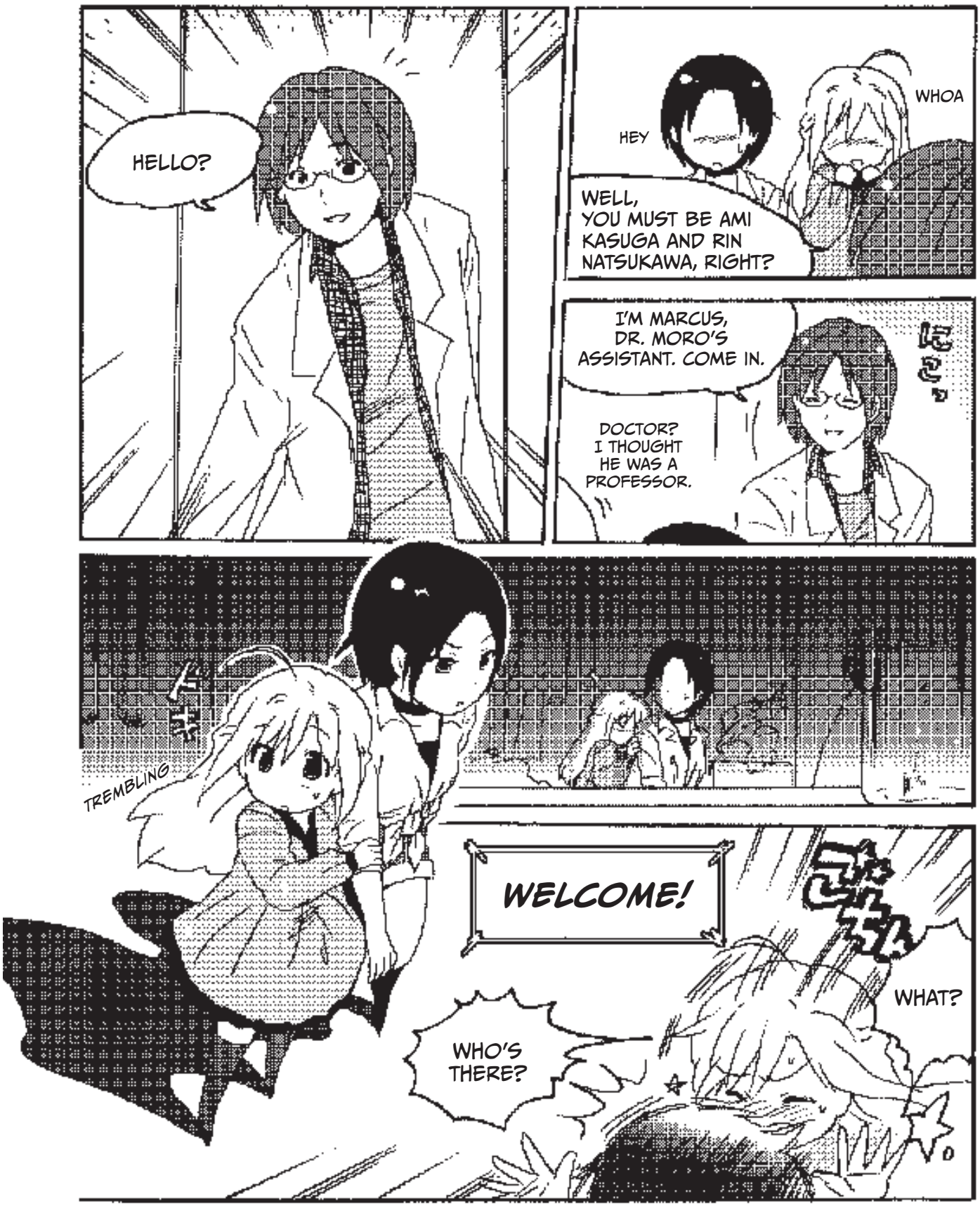

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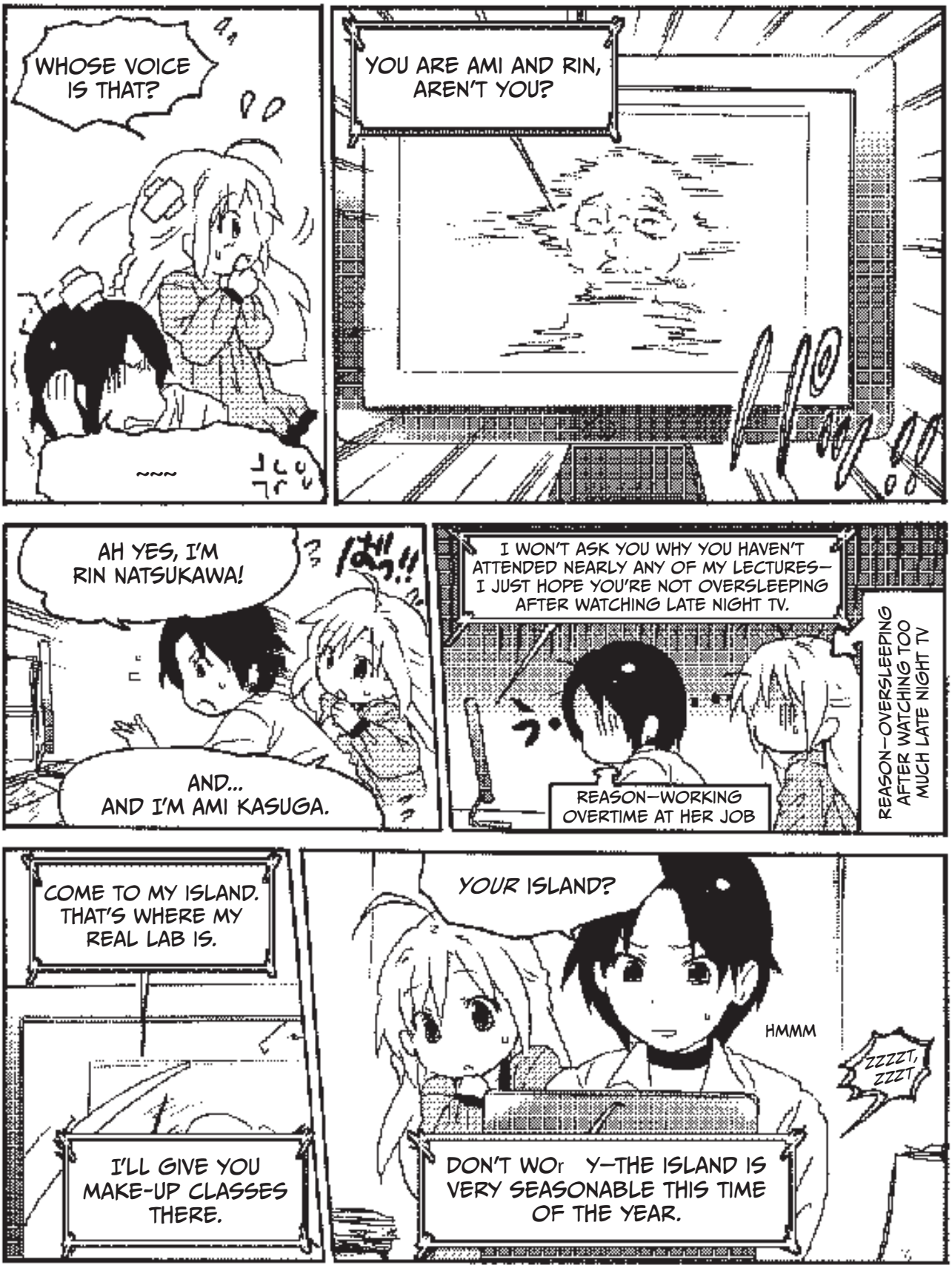

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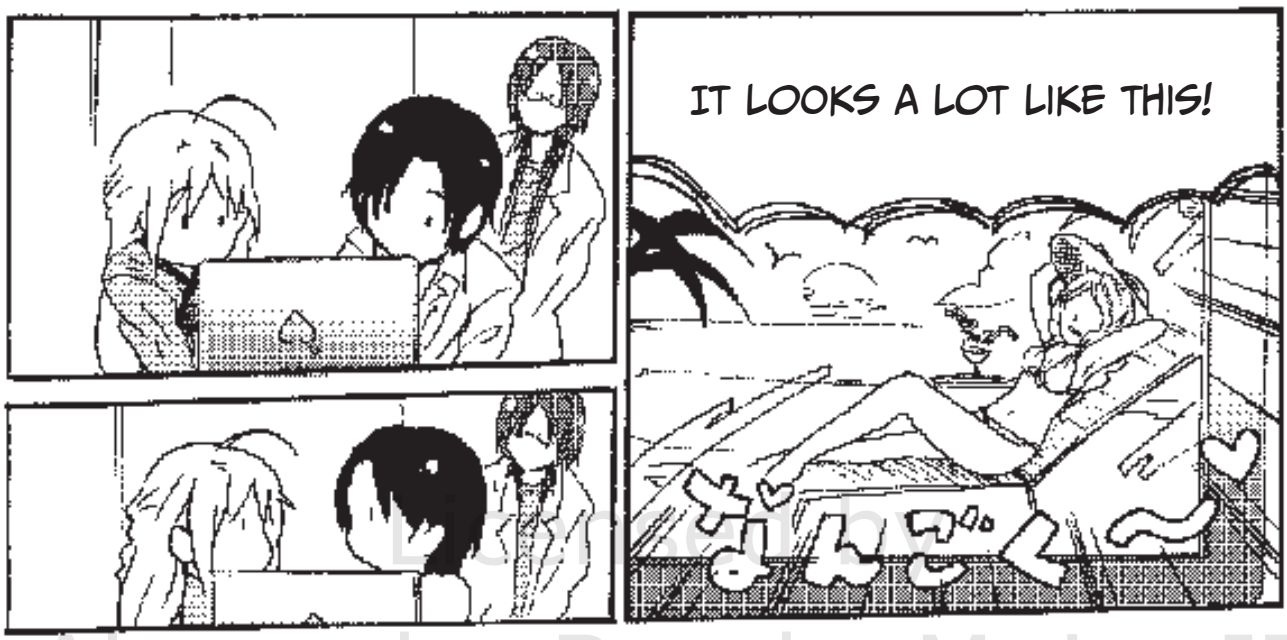

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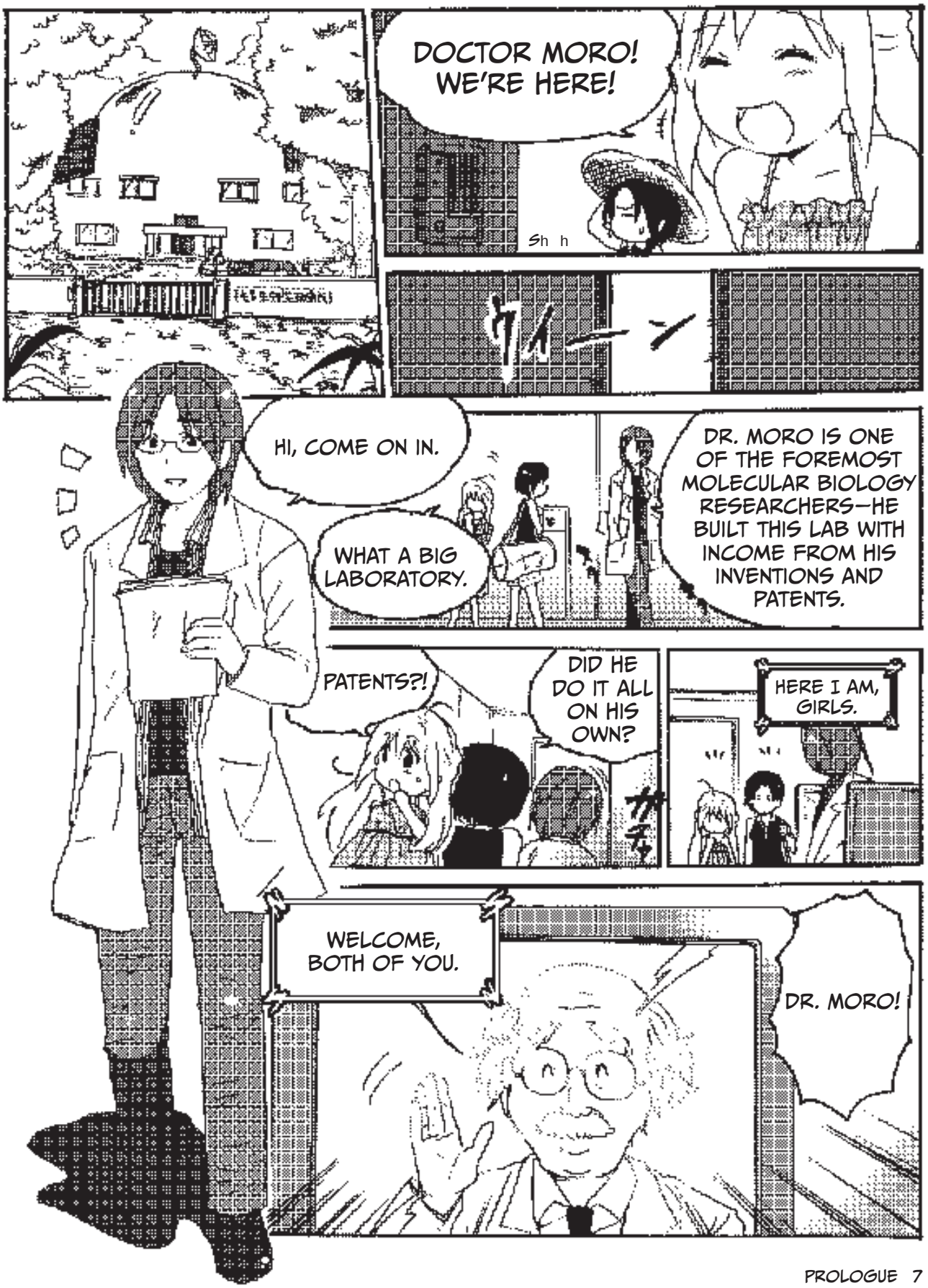

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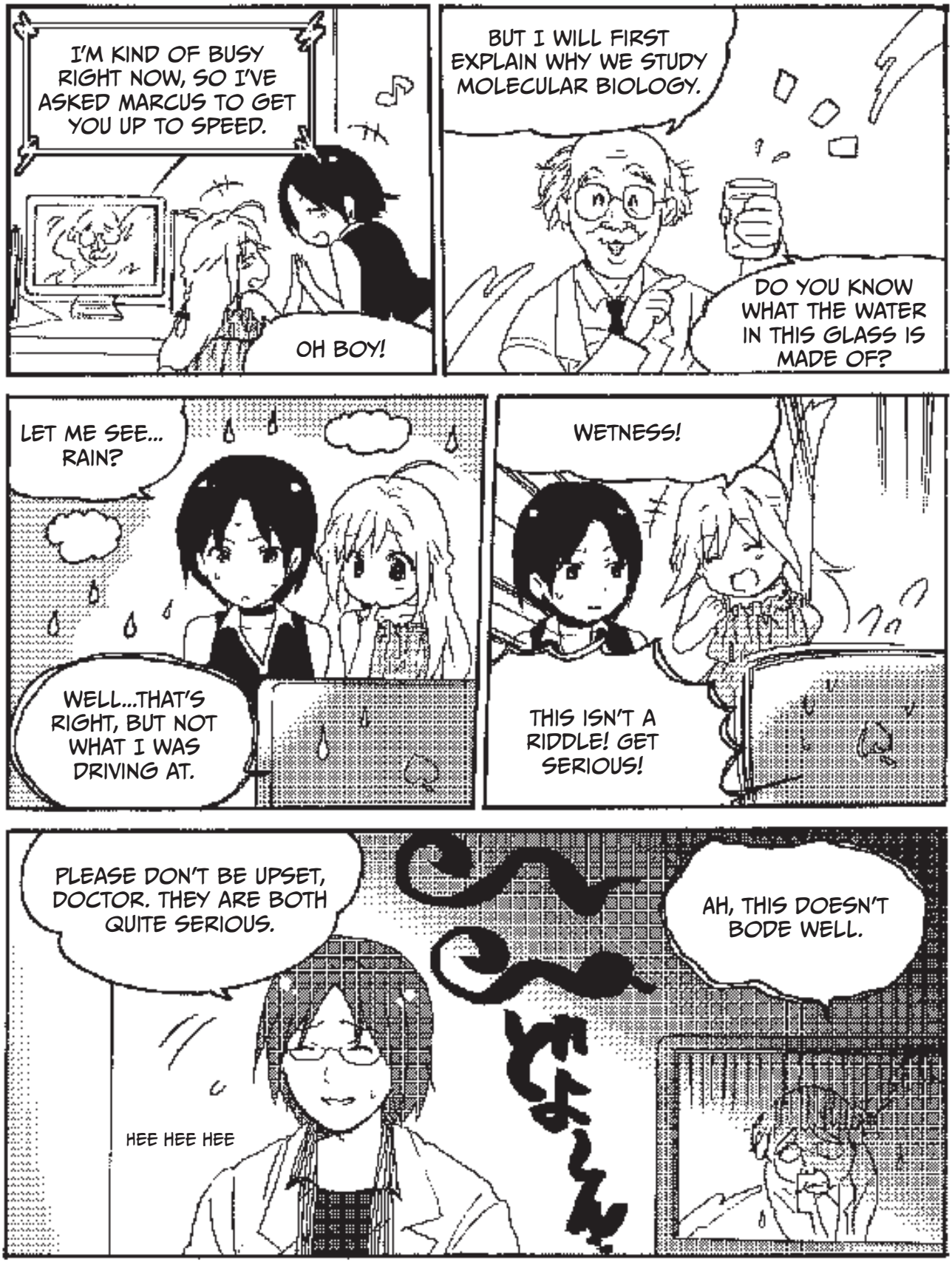

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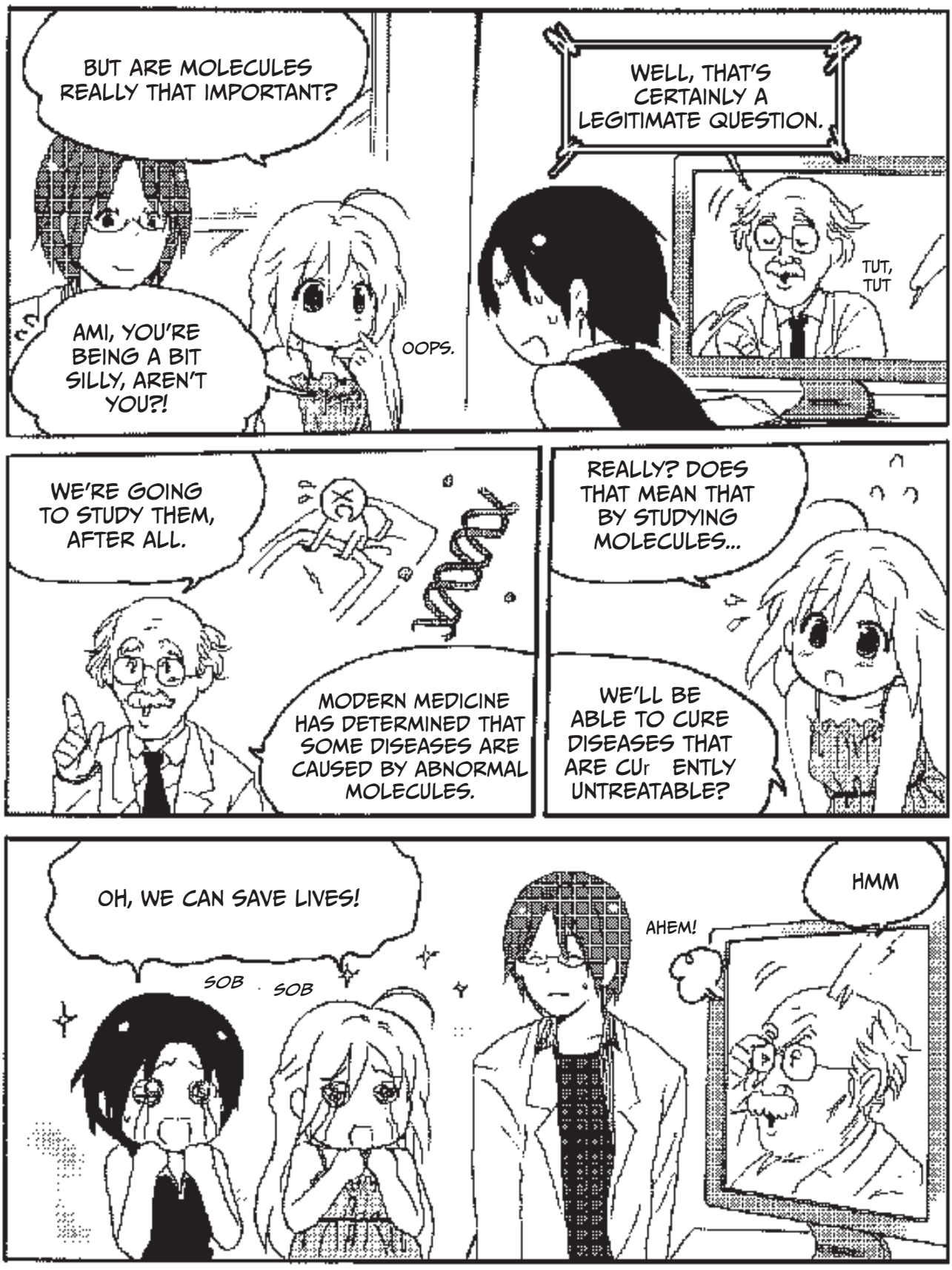

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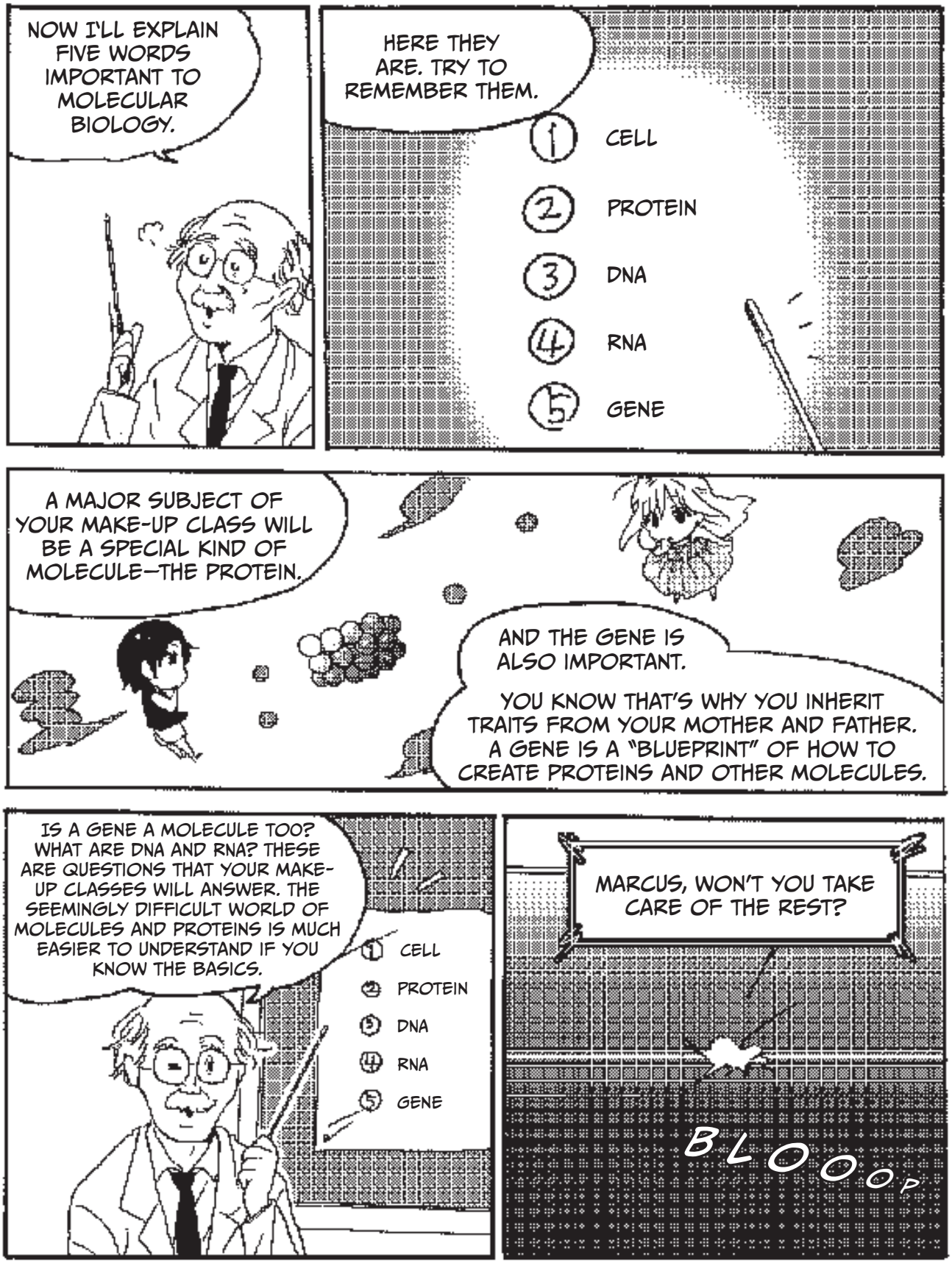

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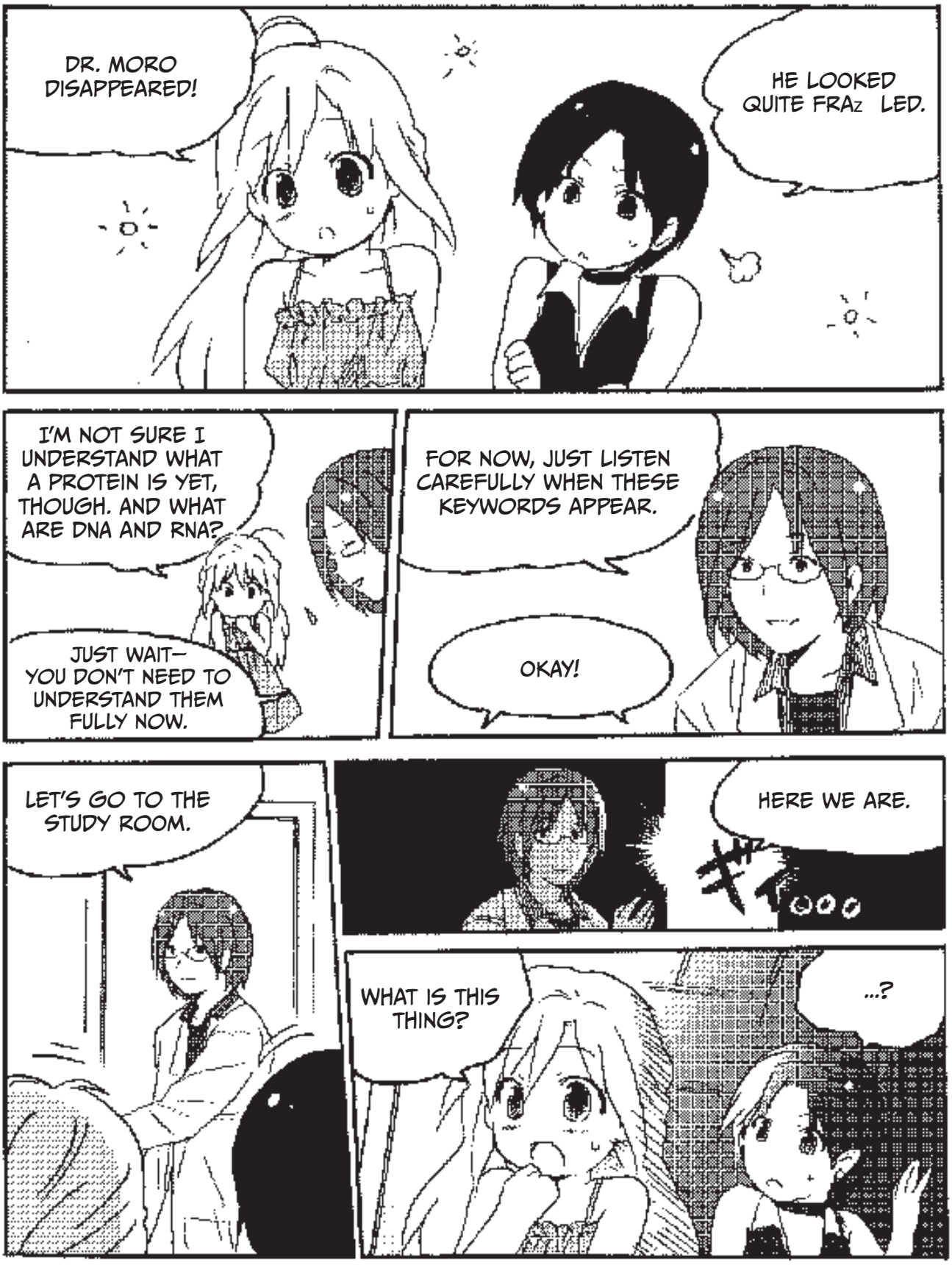

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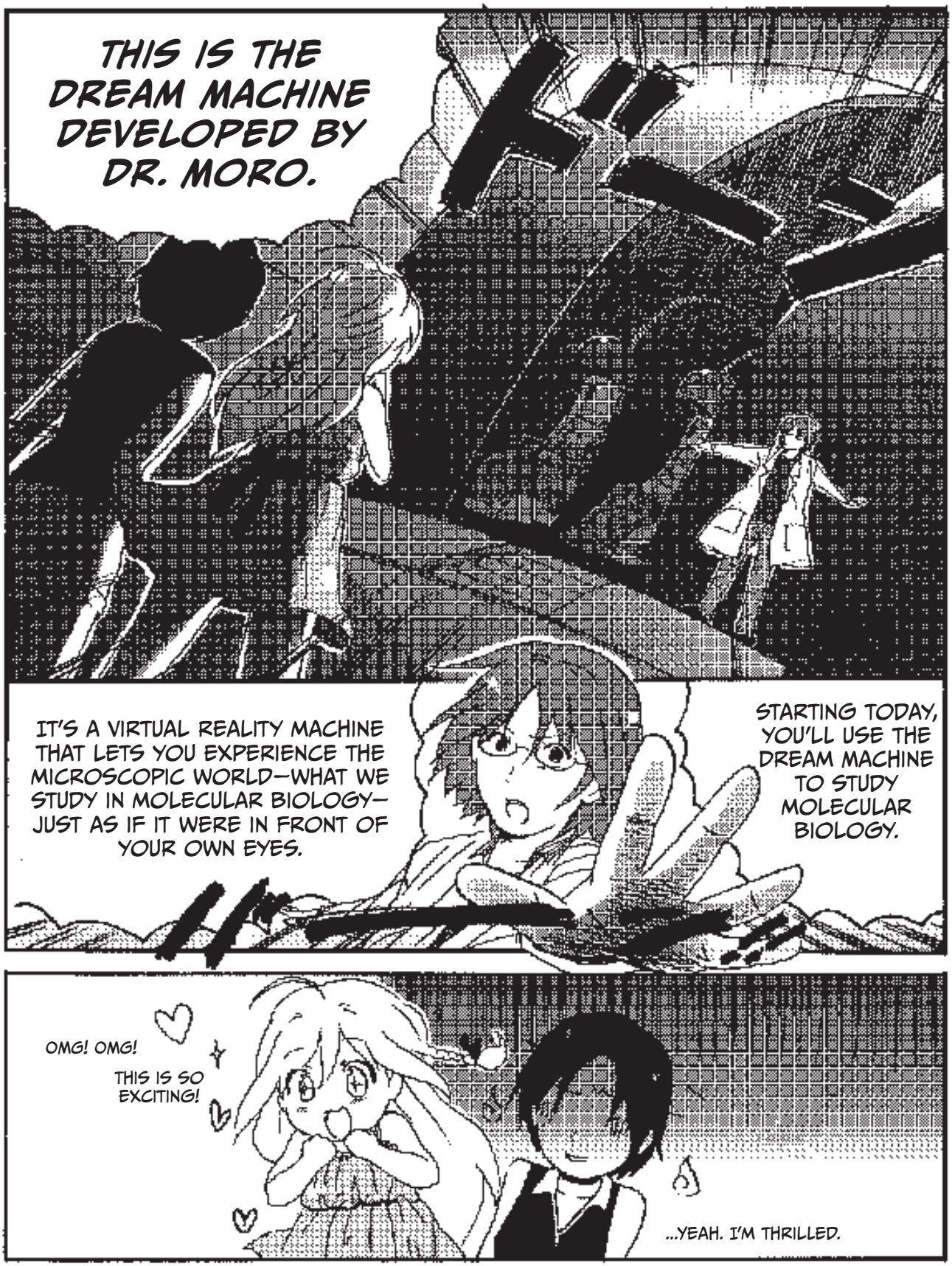

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1
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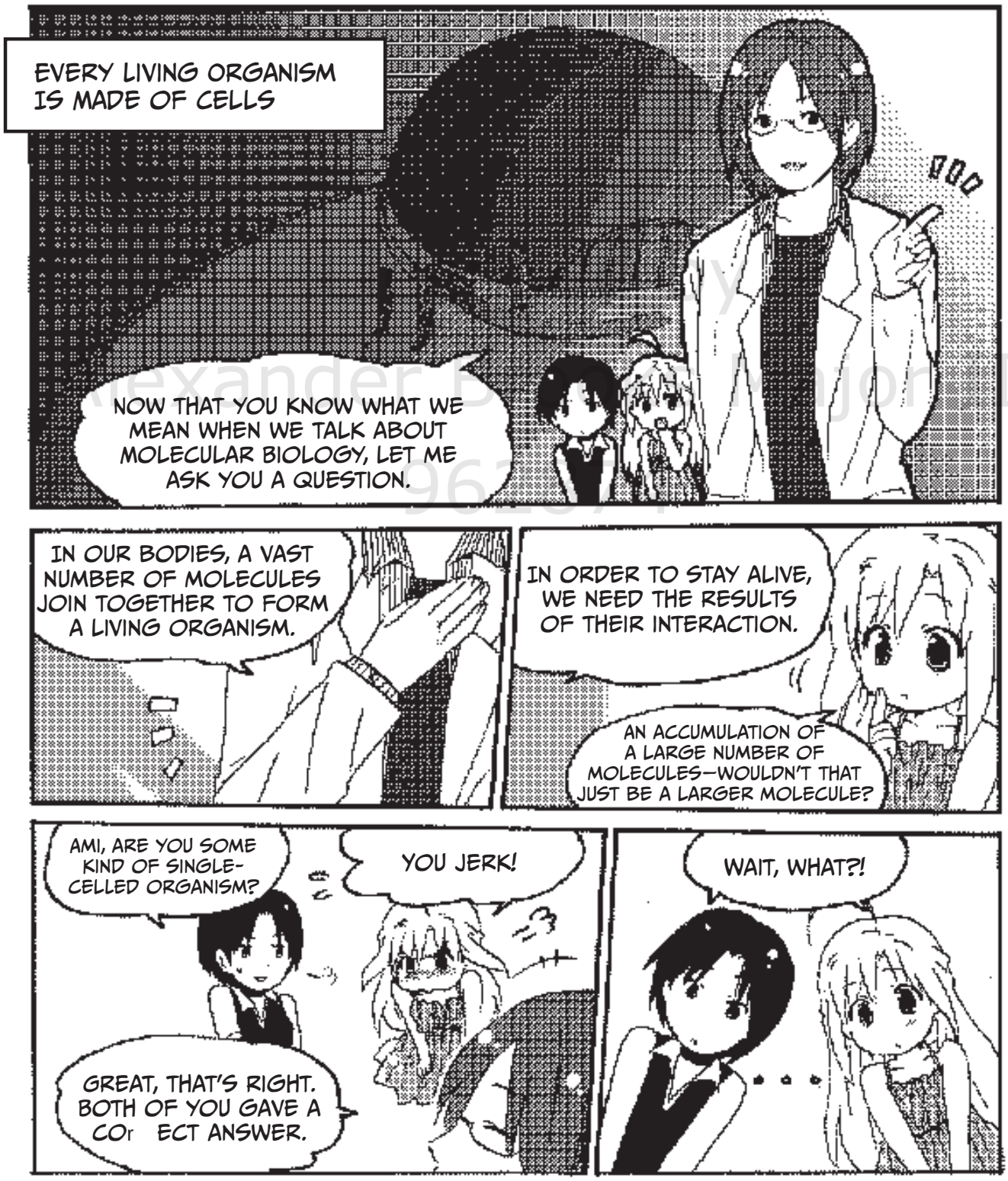
A CELL IS A LITTLE SACK OF LIFE



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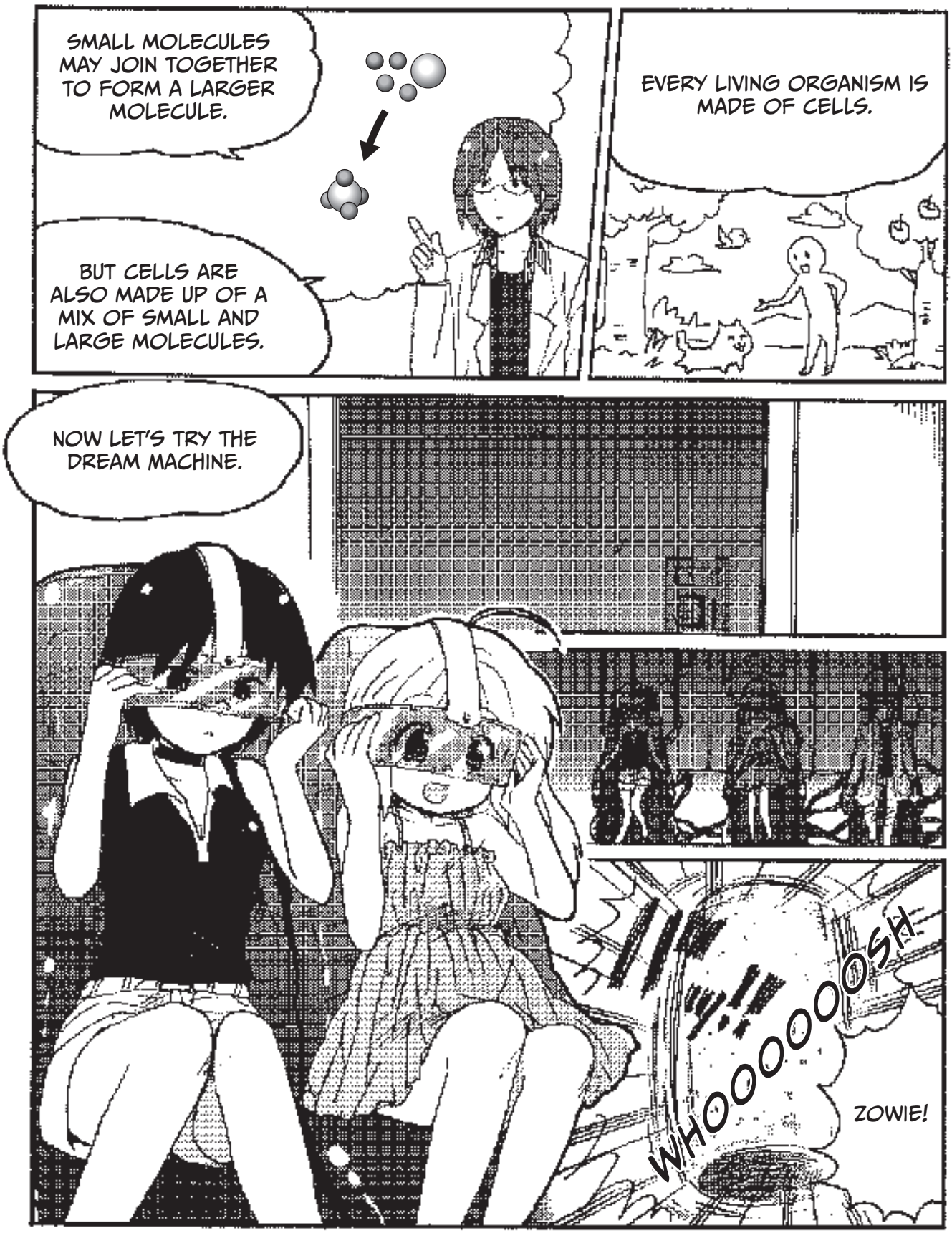

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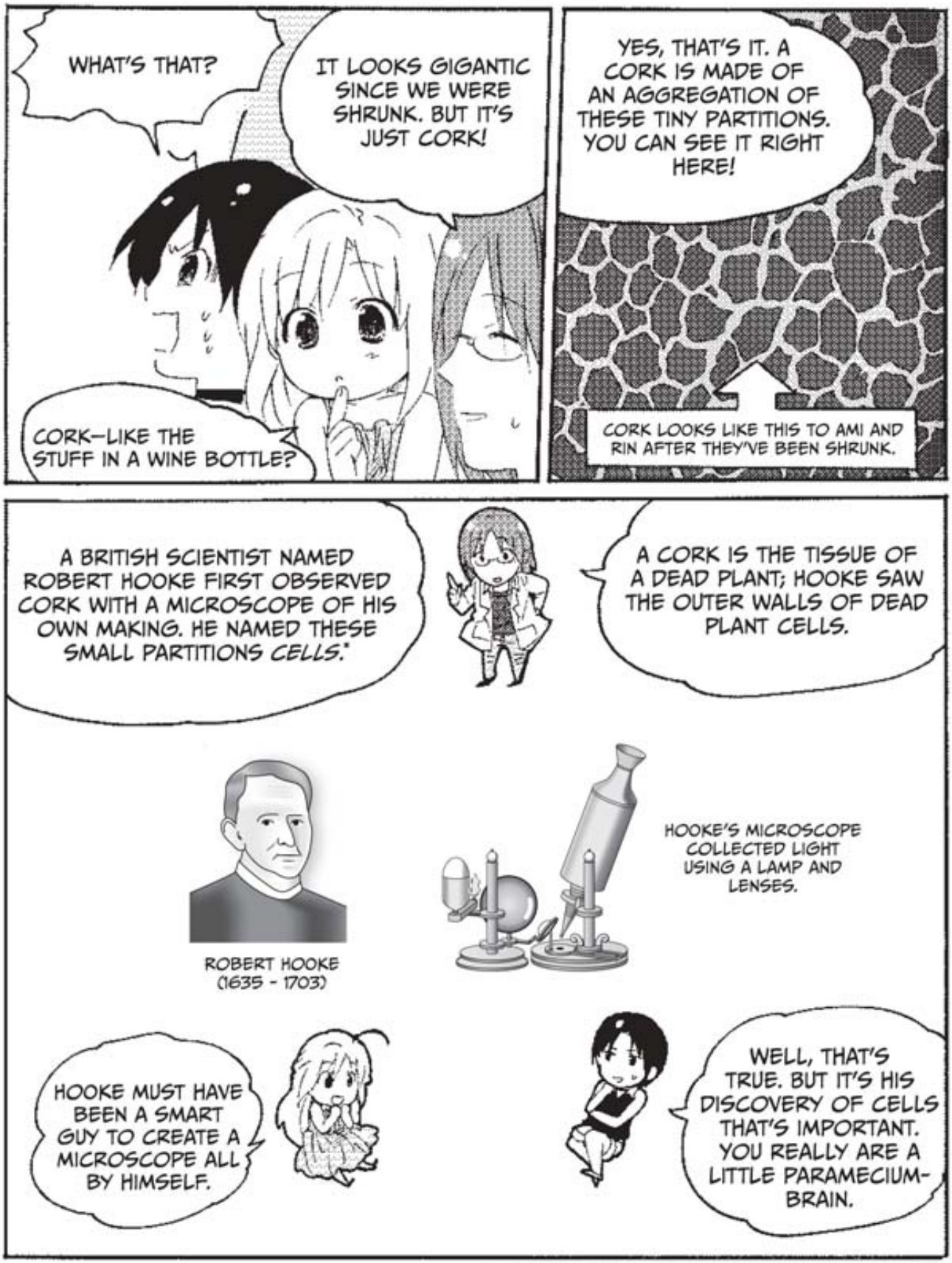

* HE CALLED THESE PARTITIONS CELLS BECAUSE THEY REMINDED HIM OF THE CELLS IN A MONASTERY—THE SMALL ROOMS WHERE MONKS LIVE.

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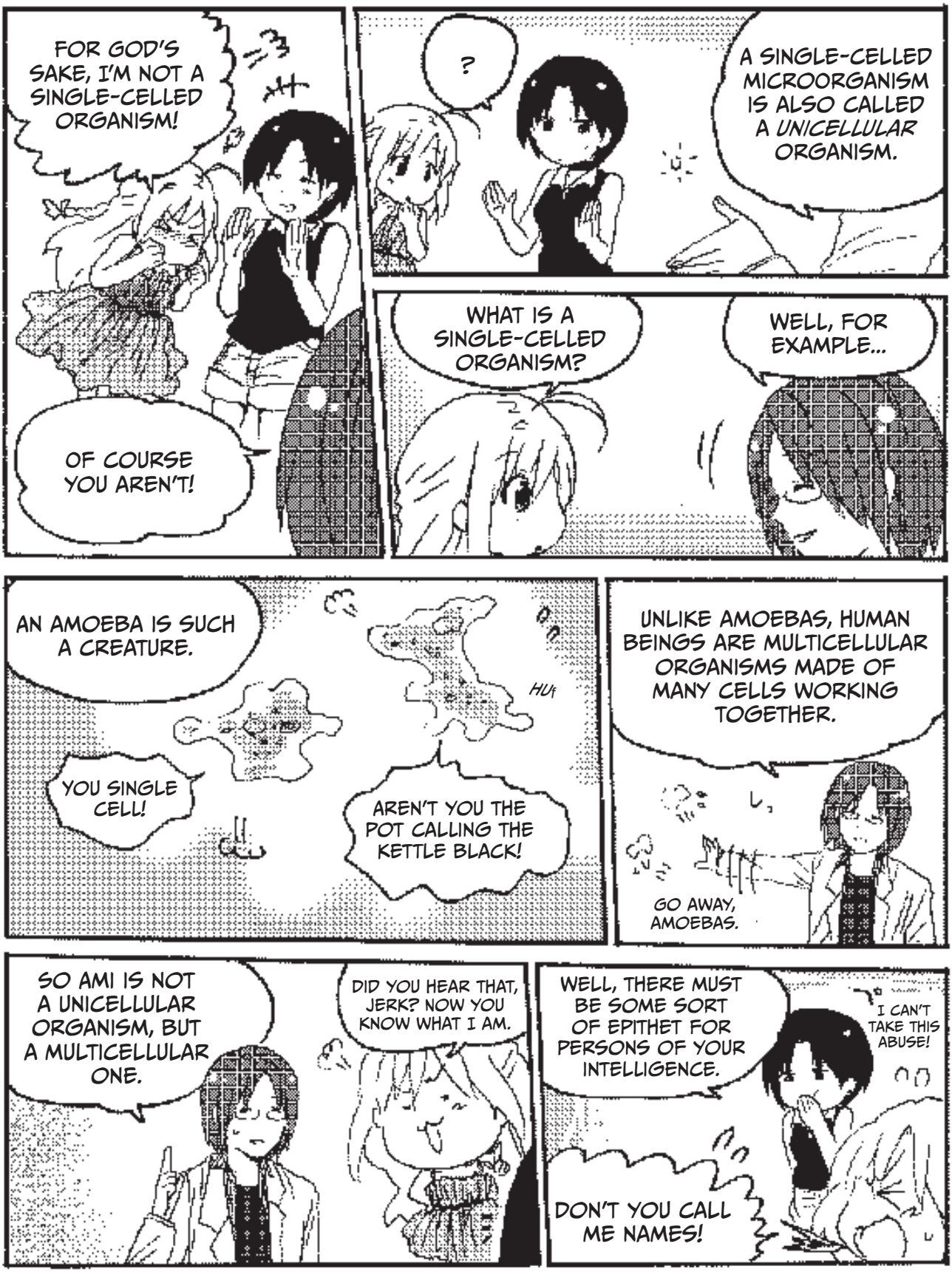

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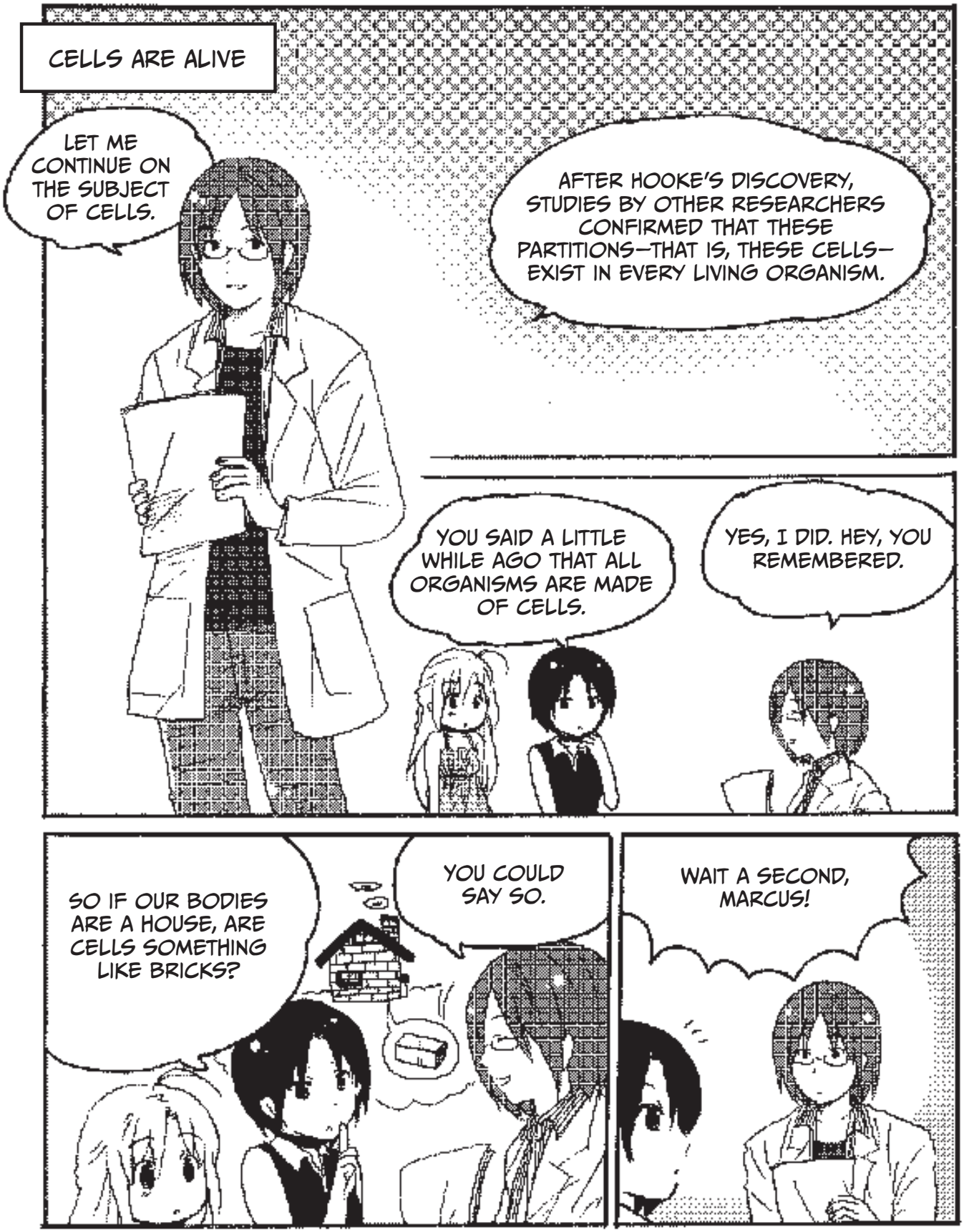

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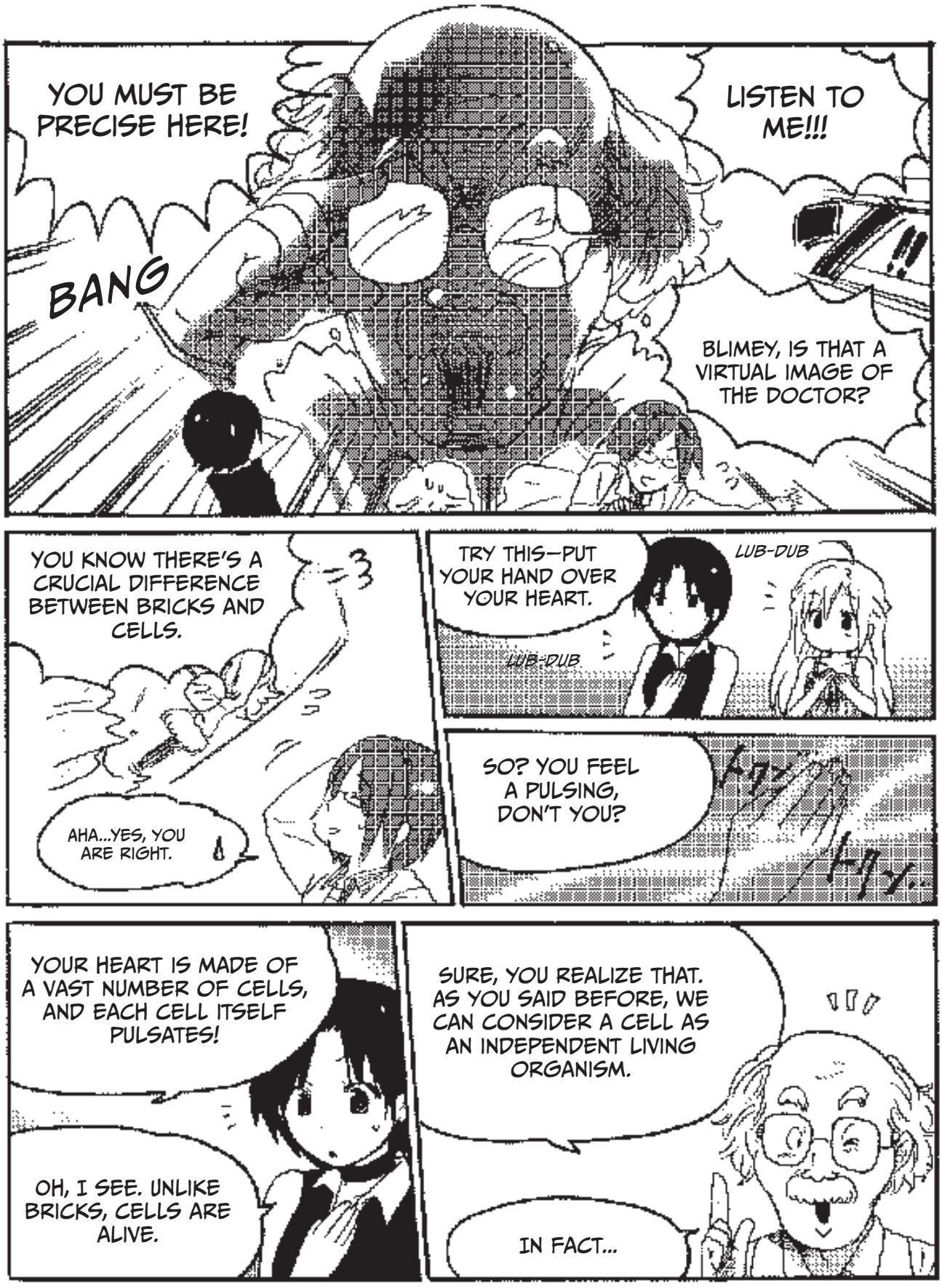

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A CELL IS MADE UP OF VARIOUS MOLECULES

A cell is a result of many different molecules acting together. A variety of molecules, large and small, react with each other to form a working "society," which we call a cell.

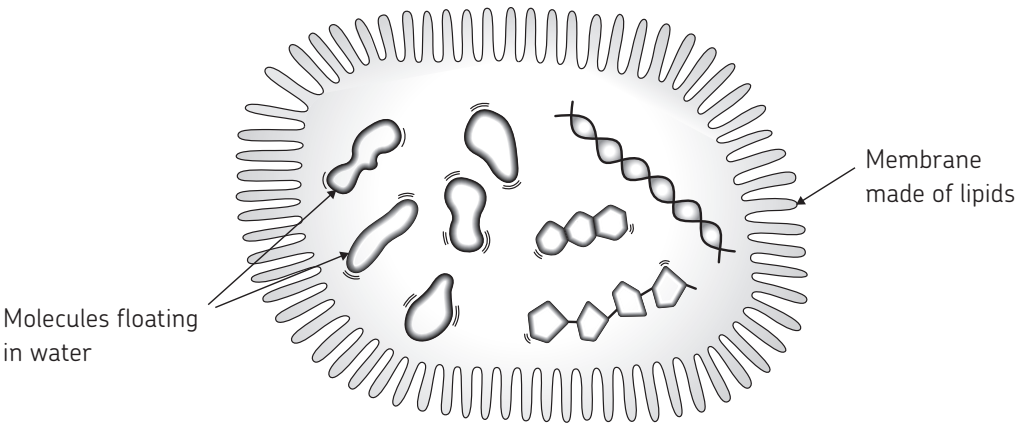
Large molecules are things like nucleic acids (such as DNA), proteins, lipids (such as fats and cholesterol), and polysaccharides (such as starch). Smaller molecules like water, amino acids, and minerals are also in cells.

Do you remember that Dr. Moro said that proteins play an important role in cellular activity?

A large protein molecule is made up of a number of linked molecules called *amino acids*, which can be subdivided into 20 types. Proteins with various properties are created, depending upon their combination. The structure of a protein determines its function. Each protein carries out its own unique work—and our cells are alive thanks to the work done by these proteins.

Now let's take a closer look at the structure of a cell. The outside of the cell is called the *cell membrane*—it's made of a fatty material called *lipids*.

At its most basic, a cell is simply a cell membrane made of lipids, with various molecules floating inside it.



A number of molecules are floating in a cell.

Glucose is also present in the cell—it is one of the most basic carbohydrates. You must have heard that rice and spaghetti are composed of carbohydrates. Glucose is contained in those foods and functions as an energy source in a cell.

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I'VE NEVER SEEN A CELL!

But wait, perhaps you have. So far I've talked about a world that's visible only through a microscope. But you probably don't have a microscope at home. So what can we do?

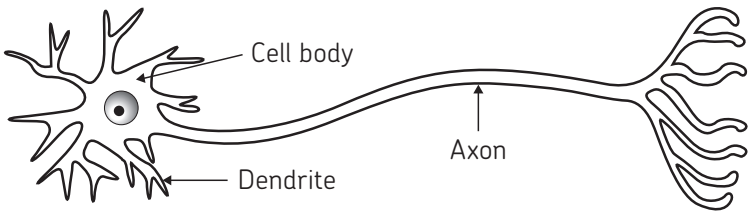
Just open the door to your refrigerator. You may see a gigantic oblong cell right there—yes, eggs, wonderful eggs! The chicken egg you eat for breakfast is just a single cell!

THE LONGEST CELL IN OUR BODIES

And of course, the human body is made up of cells, just like any other organism. At first glance, you can't find tissue that you can identify as "a single cell." But many different cells exist in our bodies, working together, as organs and other clusters.

Since we can't usually see cells without a microscope, you might think their size might be entirely microscopic. But we have a long, fine cell that's almost equivalent to the length of our bodies! This is the *nerve cell*, which responds to various stimuli, like light, sound, and touch, and is responsible for communicating these messages to the brain. Nerve cells are also known as *neurons*.

A nerve cell consists of a cell body and an axon. An *axon* is like a cable transmitting messages and stimuli through your body—and even to your brain. A number of protrusions called *dendrites* stick out from the cell body and receive messages from other neurons. The nerve cells in our body can have a meter-long axon.



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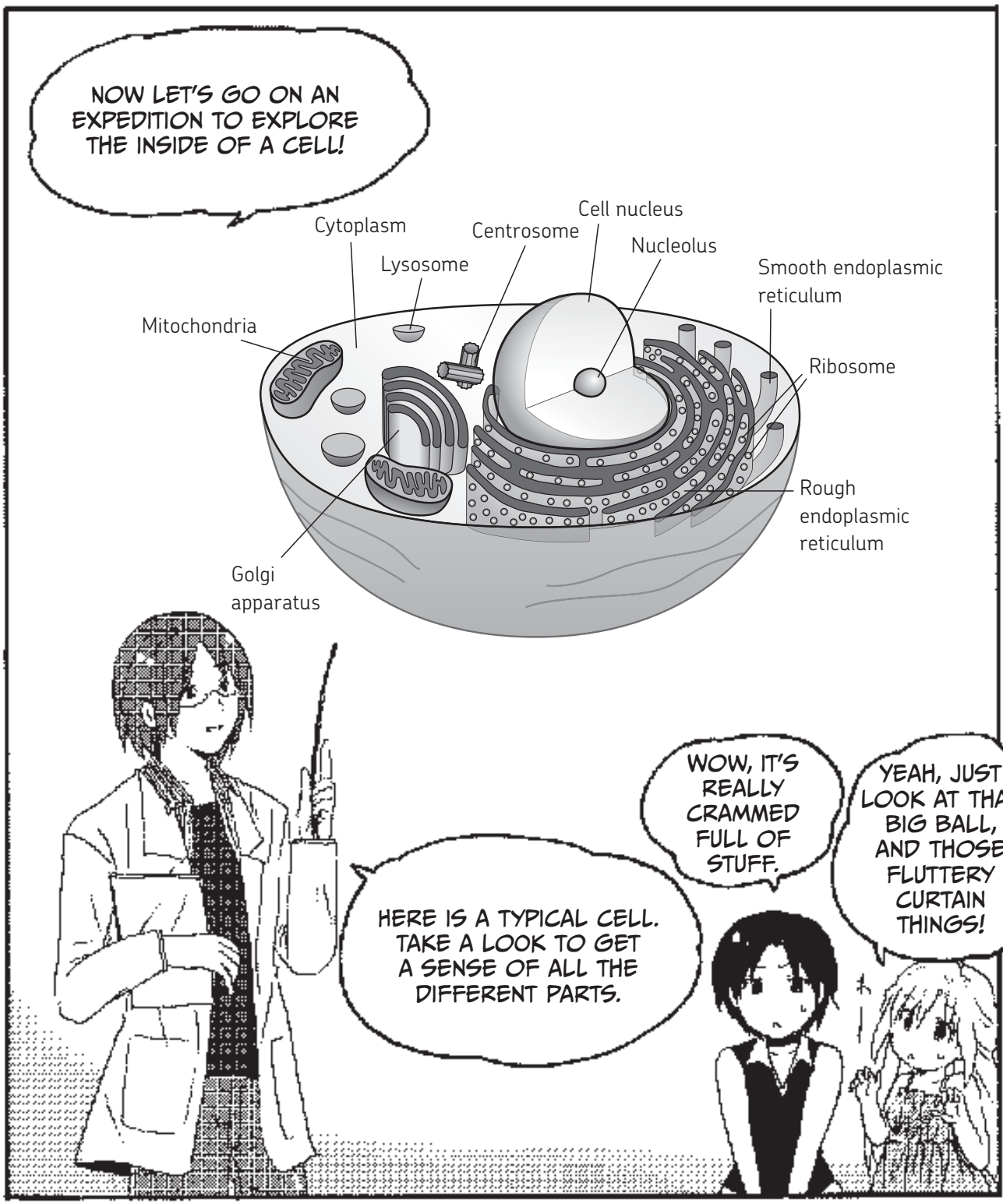
LET'S LOOK INSIDE A CELL



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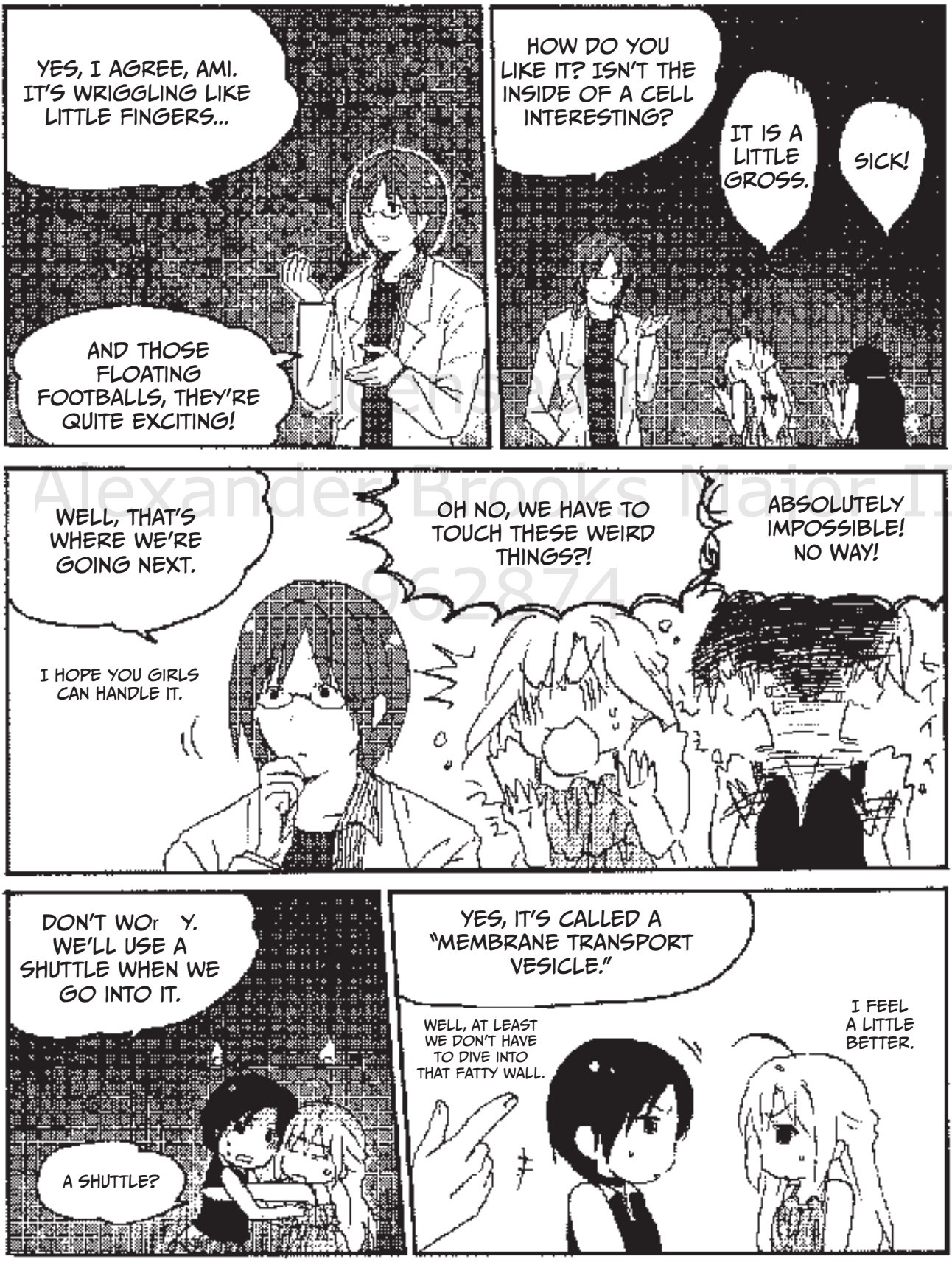

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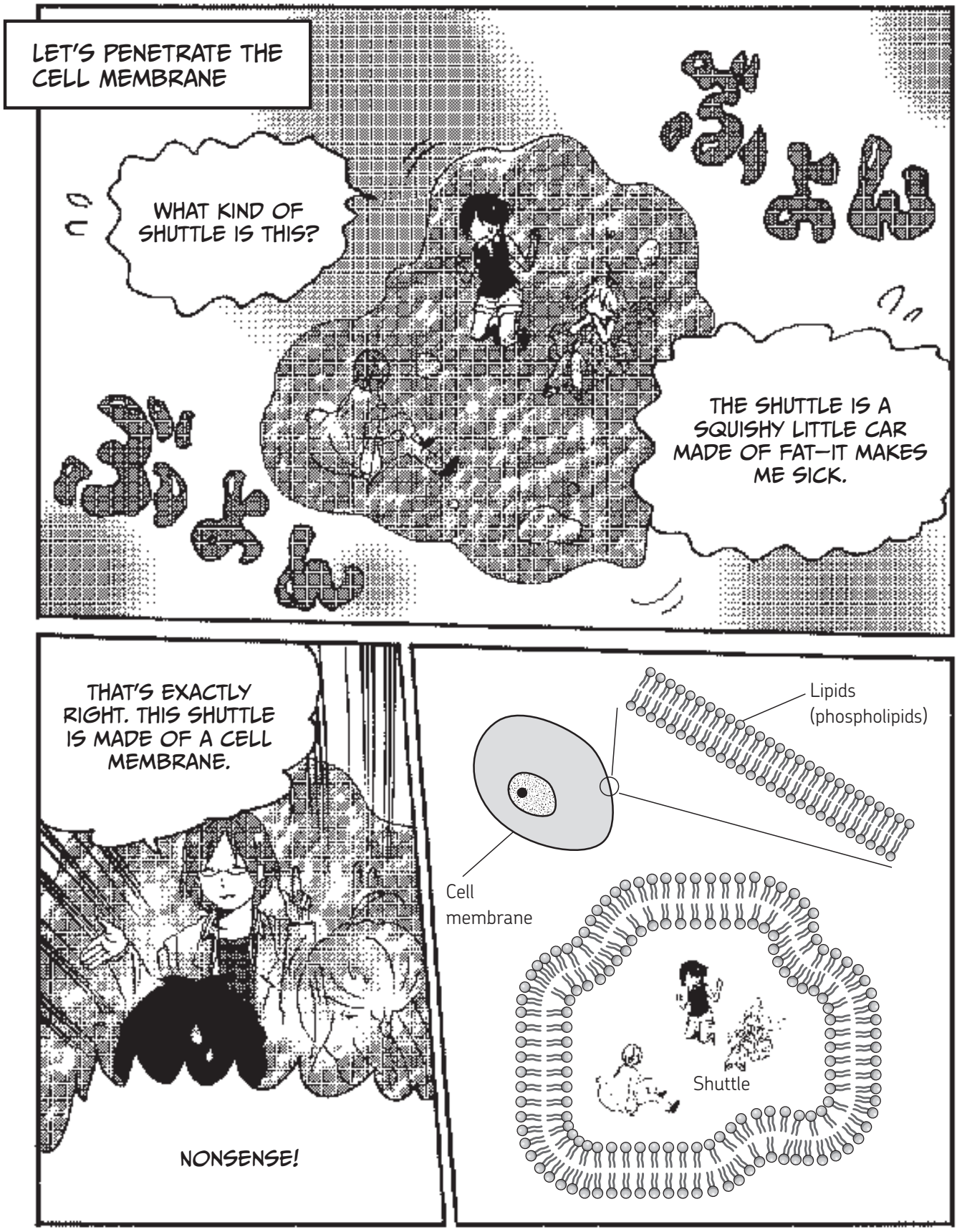

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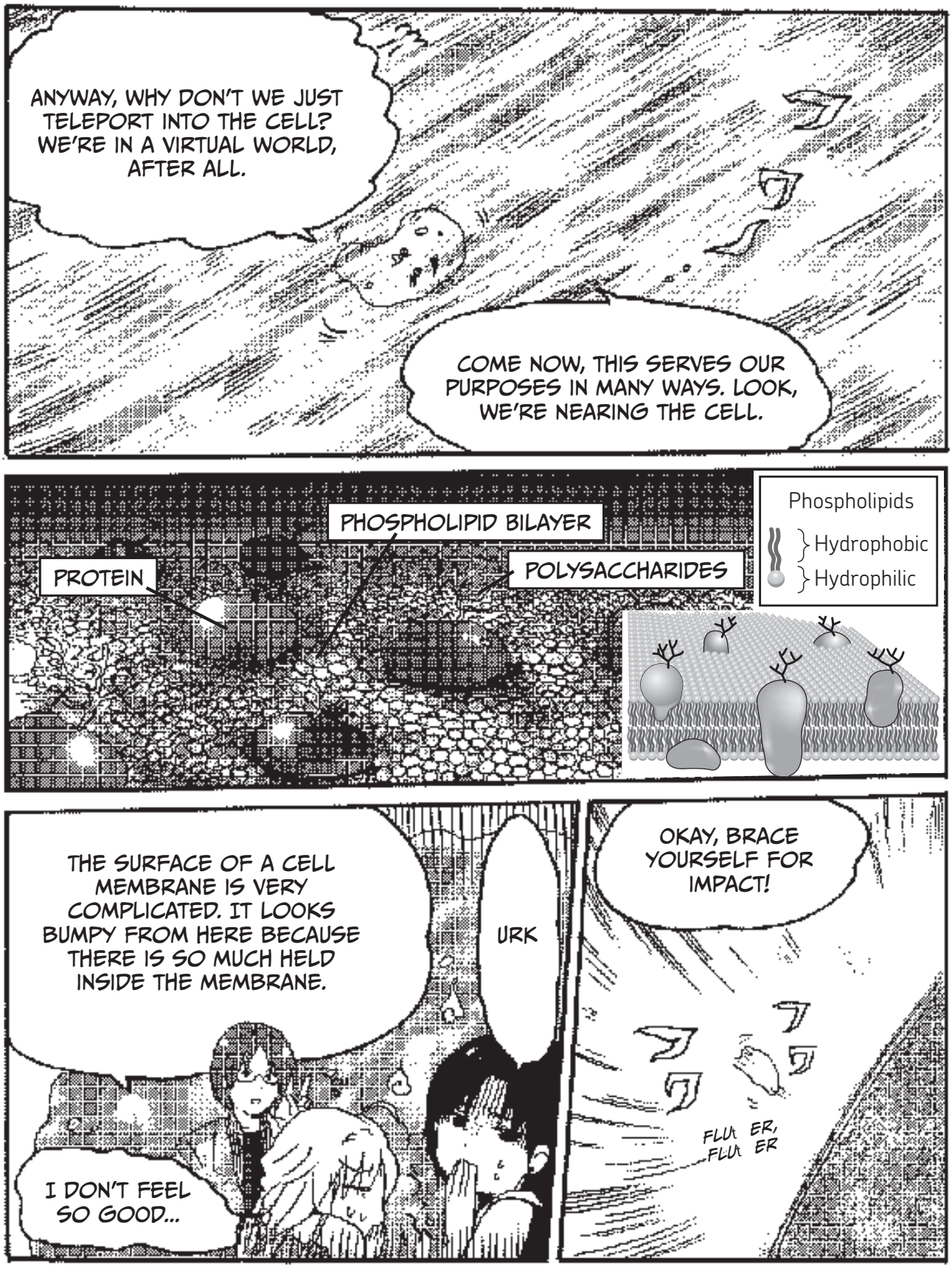

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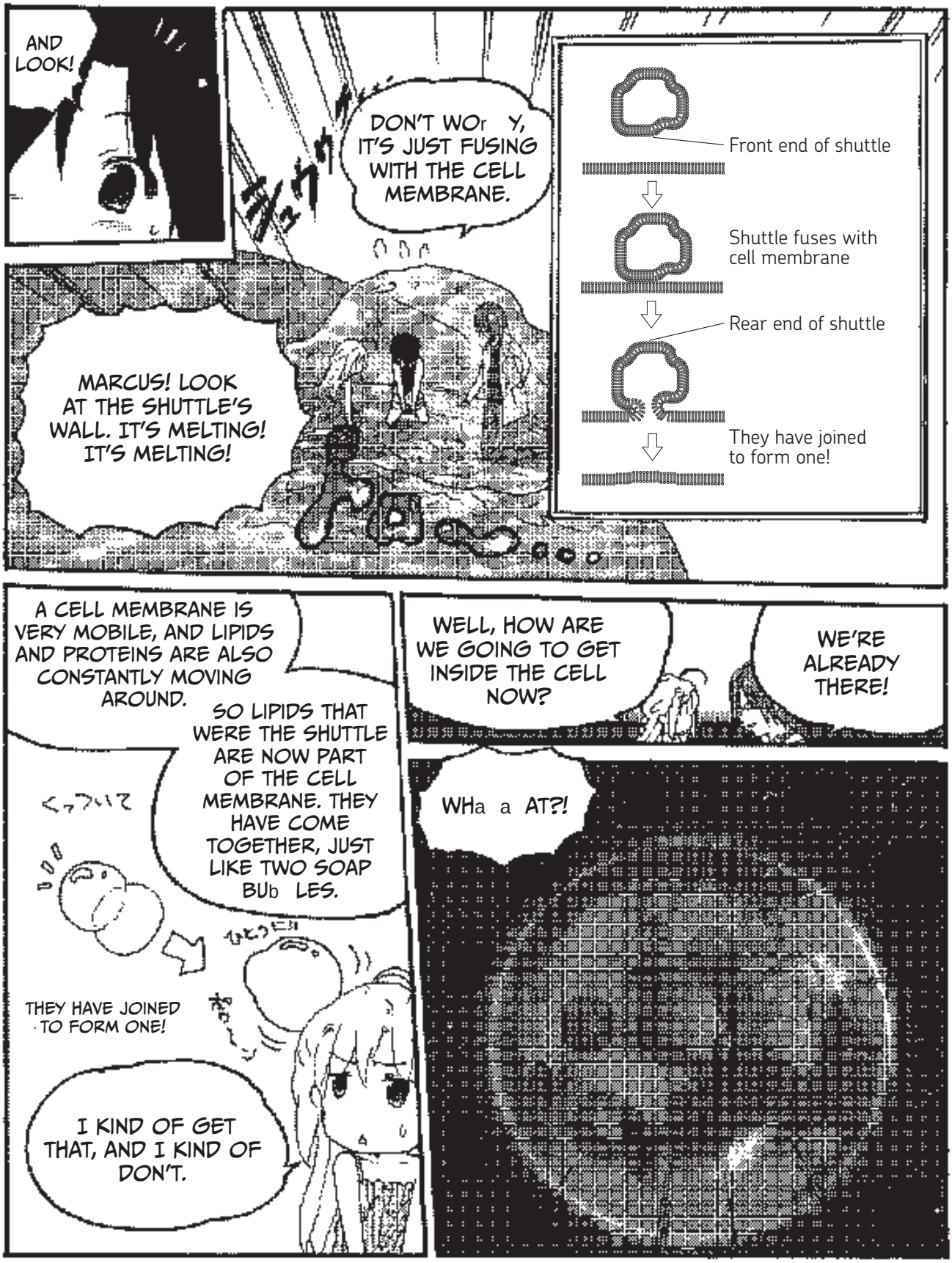

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CELL ORGANELLES

What's this gel that surrounds us?

It's *cytoplasm*, a thick solution of water and dissolved molecules. These molecules are nutrients that the cell needs and leftover waste.

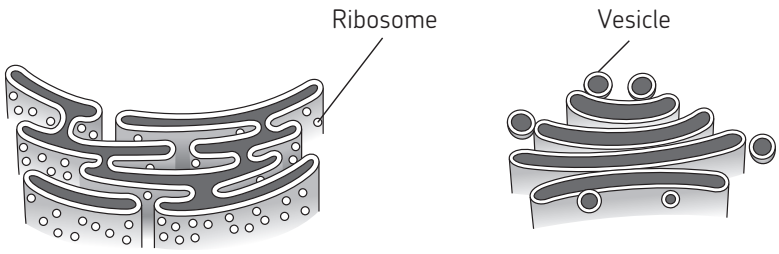
As we float through the cell's cytoplasm, we see a few larger objects floating. The cell is really packed full of these little things. Sometimes an object that resembles a giant blue whale, or a spaceship, or a giant football drifts by us.

As I mentioned, individual cells are living things and must carry out a number of tasks in order to survive. These tasks are performed by these different shapes—which are called *organelles*. Organelle means "little organ." Just like the human body has a heart, brain, and other organs that have specific duties in the body, a cell has "little organs" responsible for different tasks.

That big spaceship was actually a cell organelle.

Hey Marcus, I've been wondering for a while, what are those wall-shaped things overlapping each other in layers?

Let's move in for a closer look. The walls are made of thin films of phospholipid bilayer, just like cell membranes, but they are not actually walls. As we move closer, you can see they have a structure similar to that of a folded ribbon.



Endoplasmic reticulum

Golgi apparatus

Those ribbons are overlapping!

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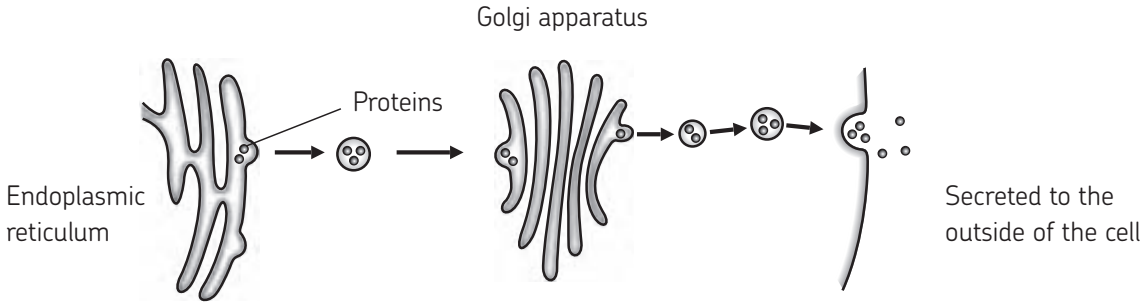
It's a cell organelle called the *endoplasmic reticulum*. Its surface is covered with many ribosomes, another smaller organelle. These two types of organelles work together to synthesize and process proteins.

Actually, there are many places in the body, like the liver and lymph nodes, where the cells make proteins specifically to be secreted for use by other cells in the body. When these proteins are secreted, a cell organelle called the *Golgi apparatus* functions as a delivery center. The process for packaging and delivering proteins is just like the way we entered the cell—but in reverse! The Golgi apparatus is also known as a Golgi body. Golgi bodies package proteins and other molecules into membrane bags, called *vesicles*. These vesicles deliver molecules made inside the cell to the outside; they can also deliver molecules to other organelles in the cells, like lysosomes, via membrane fusion.



The Golgi apparatus secretes proteins outside the cell.

Aha! That's why you carried us on such an unusual shuttle.

That's right—we entered the cell through membrane fusion.

Look, bags of cell membrane are used all over the cell to perform different functions. In a cell organelle called a *lysosome*, large molecules are broken down into smaller sizes. It functions like the digestive system of a cell, by degrading molecules.

There are also bag-like vesicles called *peroxisomes* everywhere inside the cell. They oxygenate harmful substances like bacteria, in order to neutralize them.

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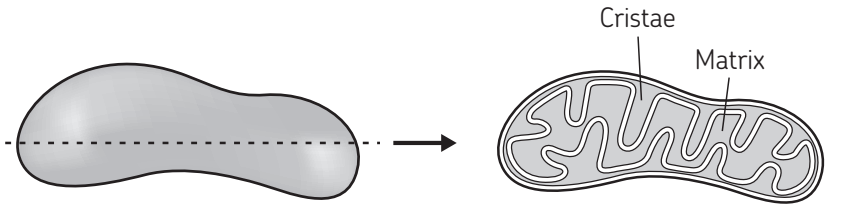
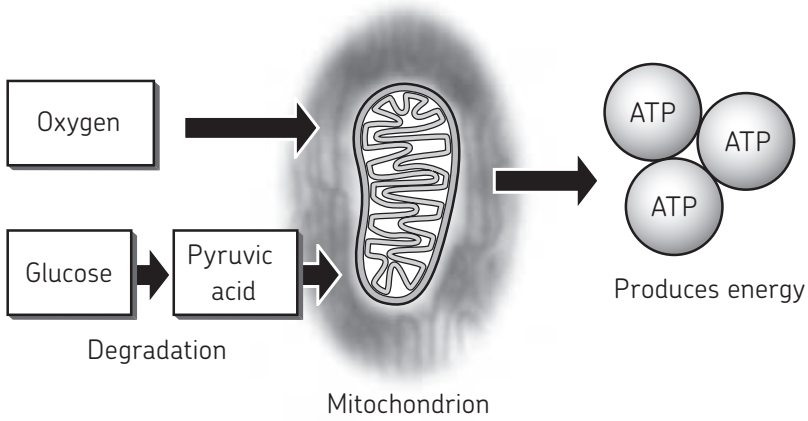
Mitochondria are also very important cell organelles. They are a sort of "power plant" for producing the energy necessary for cells to live. Cells need mitochondria to survive!



Mitochondria

Mitochondria produce the energy a cell needs through a chemical substance called *ATP*. It creates ATP using oxygen taken into our body through aspiration (breathing) and pyruvic acid, a product made from the breakdown of glucose (sugar).



Power plant

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Well, I think I can remember Golgi apparatus and mitochondria, but ribosome, lysosome, and peroxisome sound similar and are difficult to remember.

Yes, people often get these names mixed up. But if you know a little bit about Greek, you might remember their names better. *Some* means body, like a bag. *Lyso* means to break down. So it makes sense that a lysosome is a bag-like organelle that breaks down molecules. In the same way, *peroxi* means oxygen. And if you can remember that you use hydrogen peroxide to clean a cut, you can remember that a *peroxisome* is an organelle that uses oxygen to kill bacteria and other dangerous things.

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THE NUCLEUS: A LITTLE BRAIN

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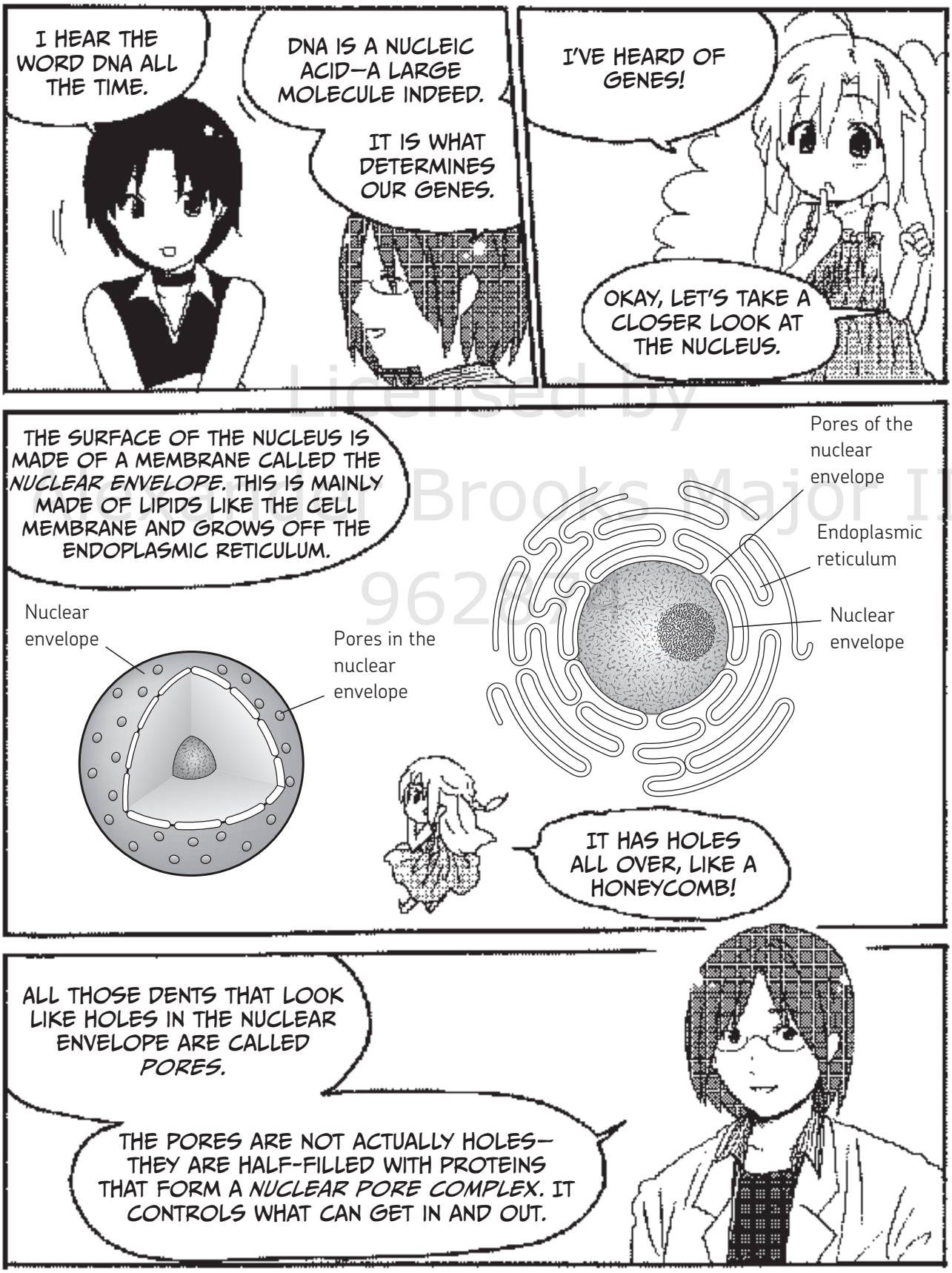

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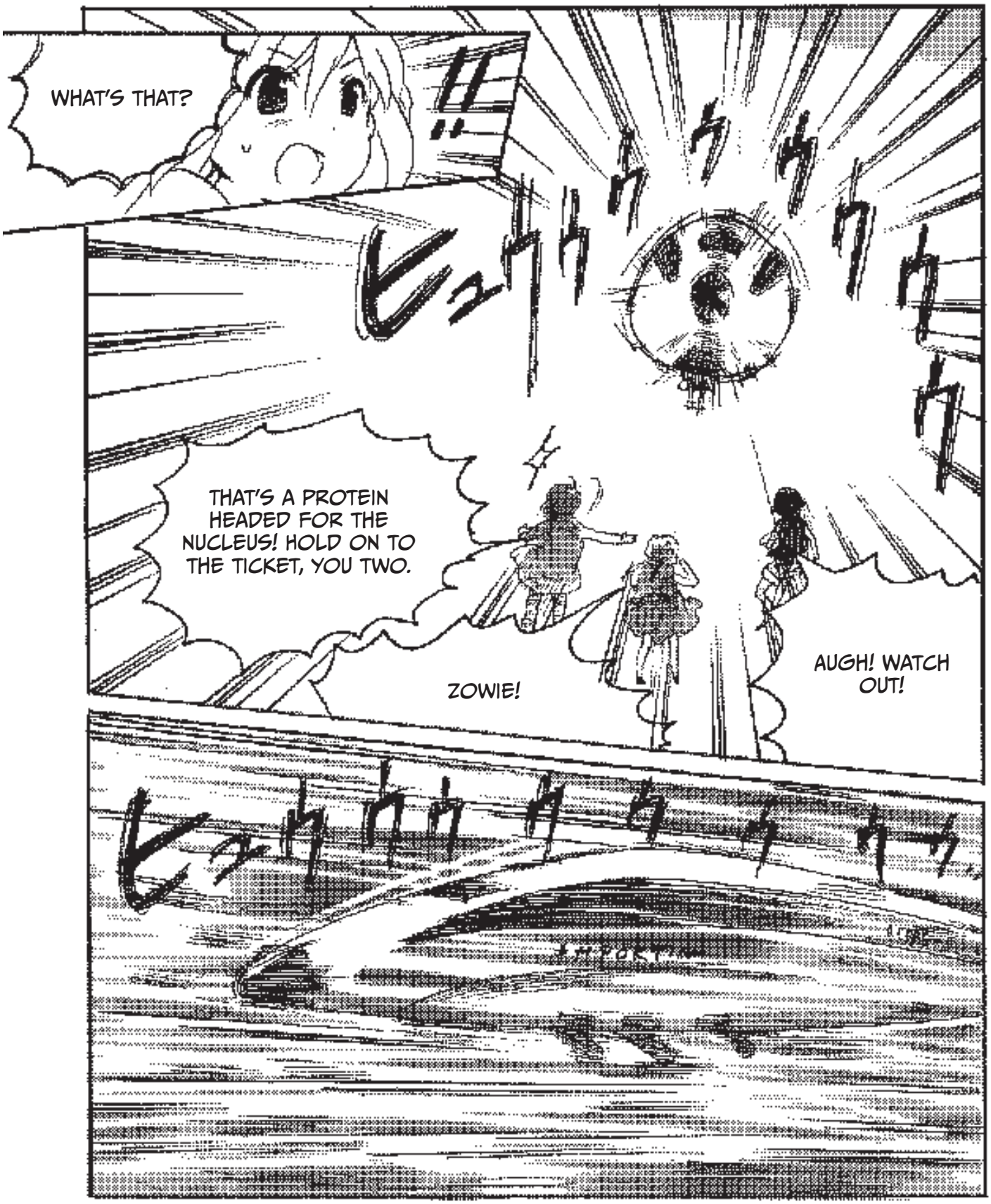

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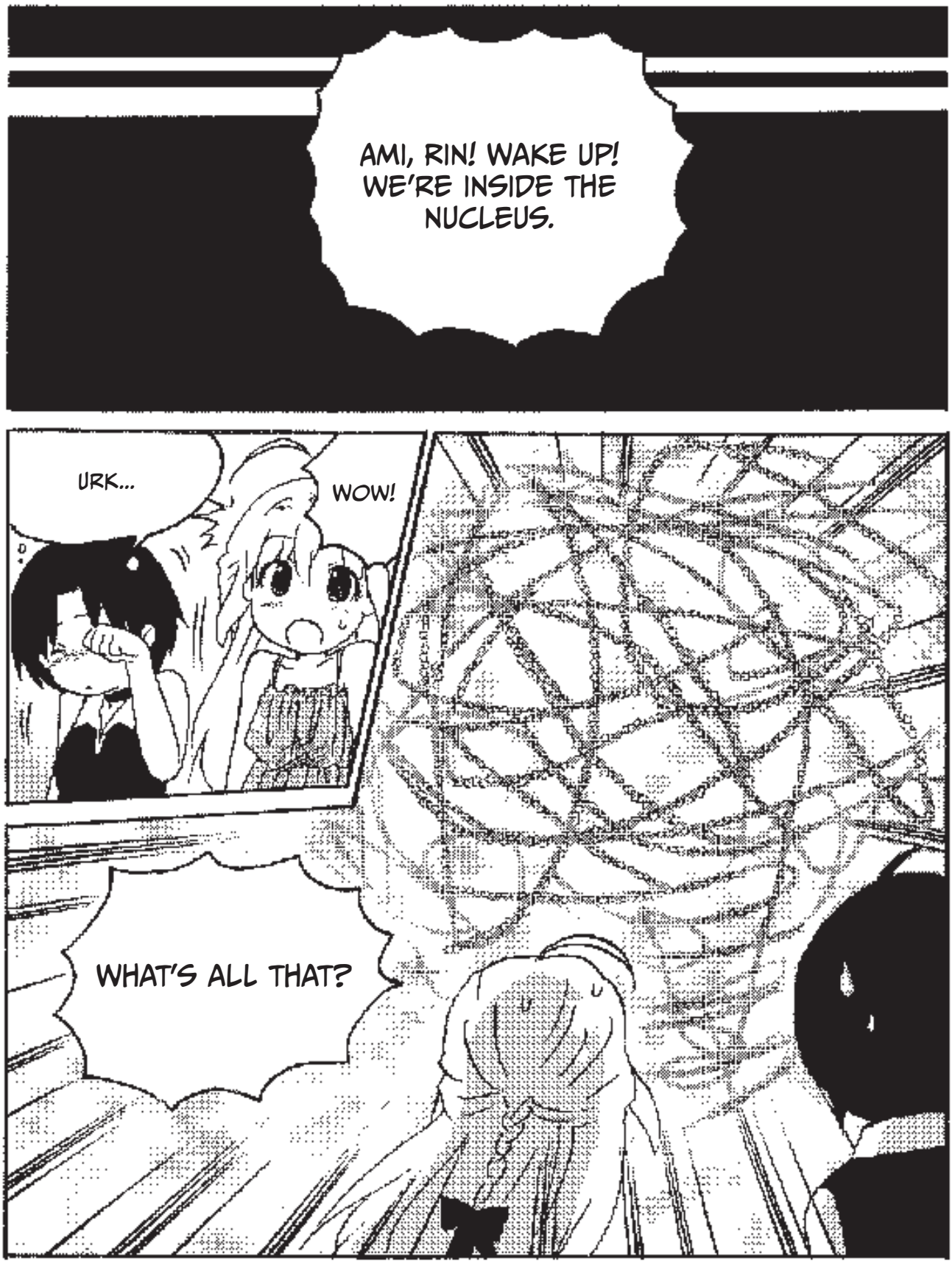

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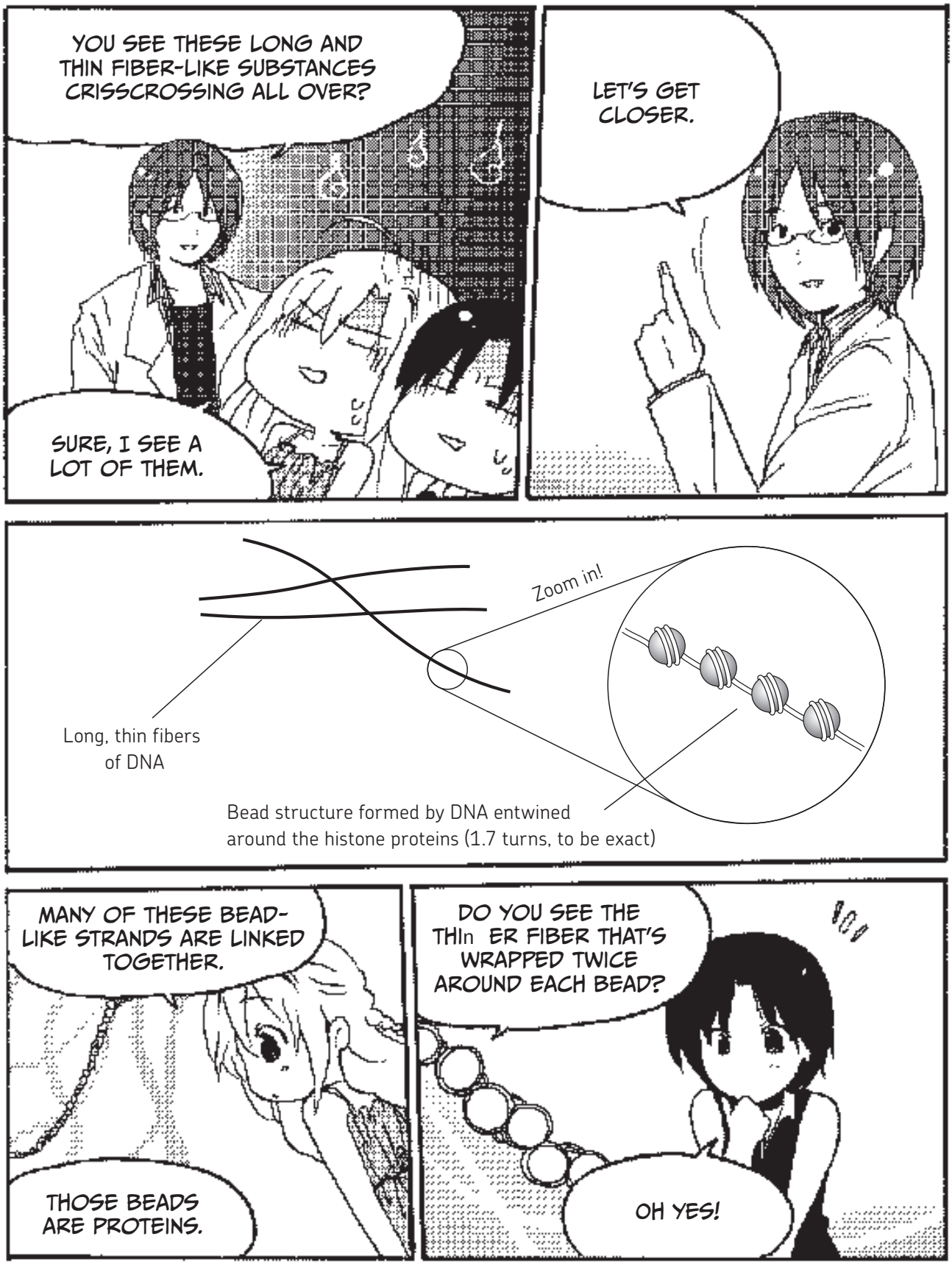

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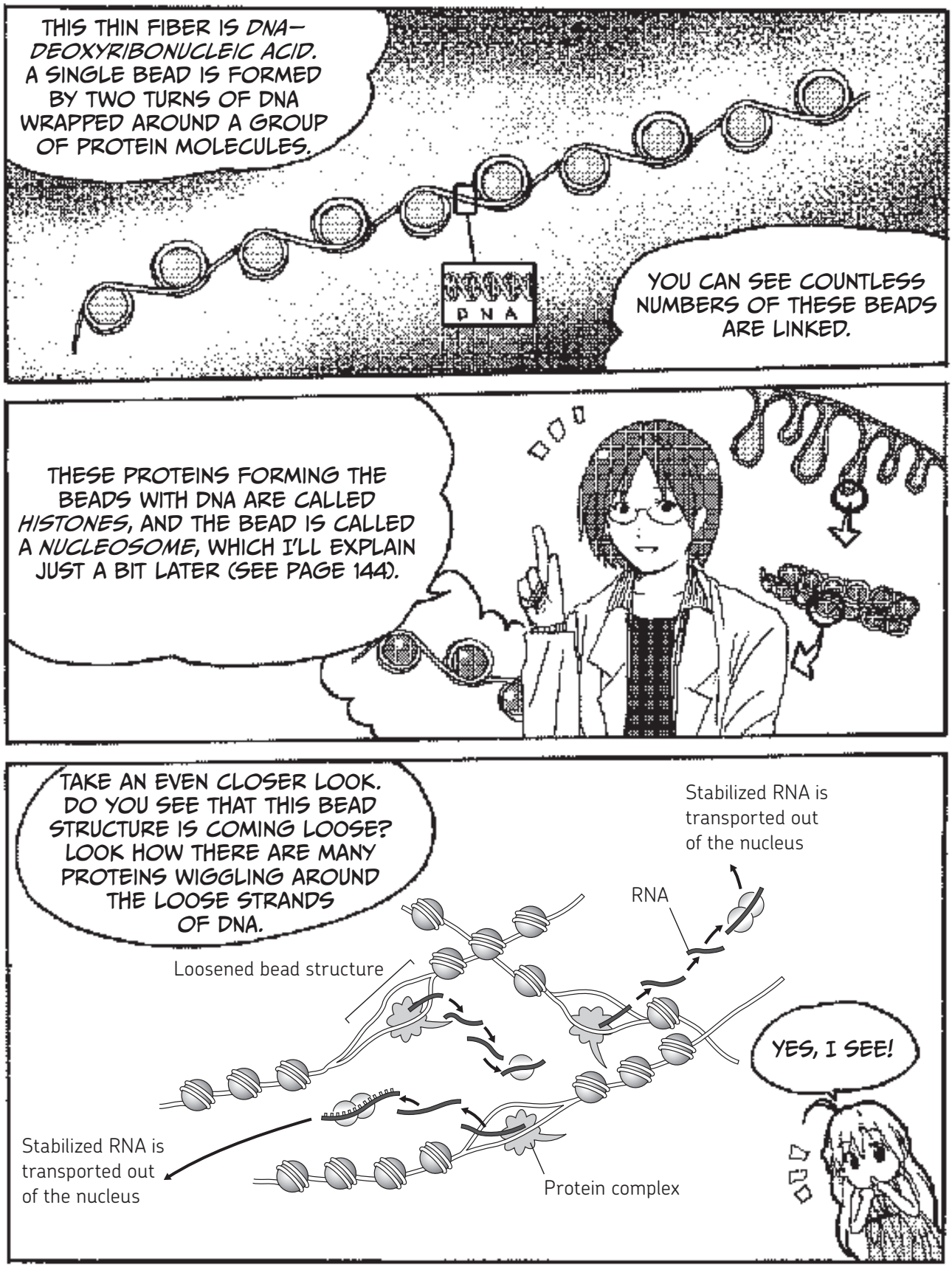

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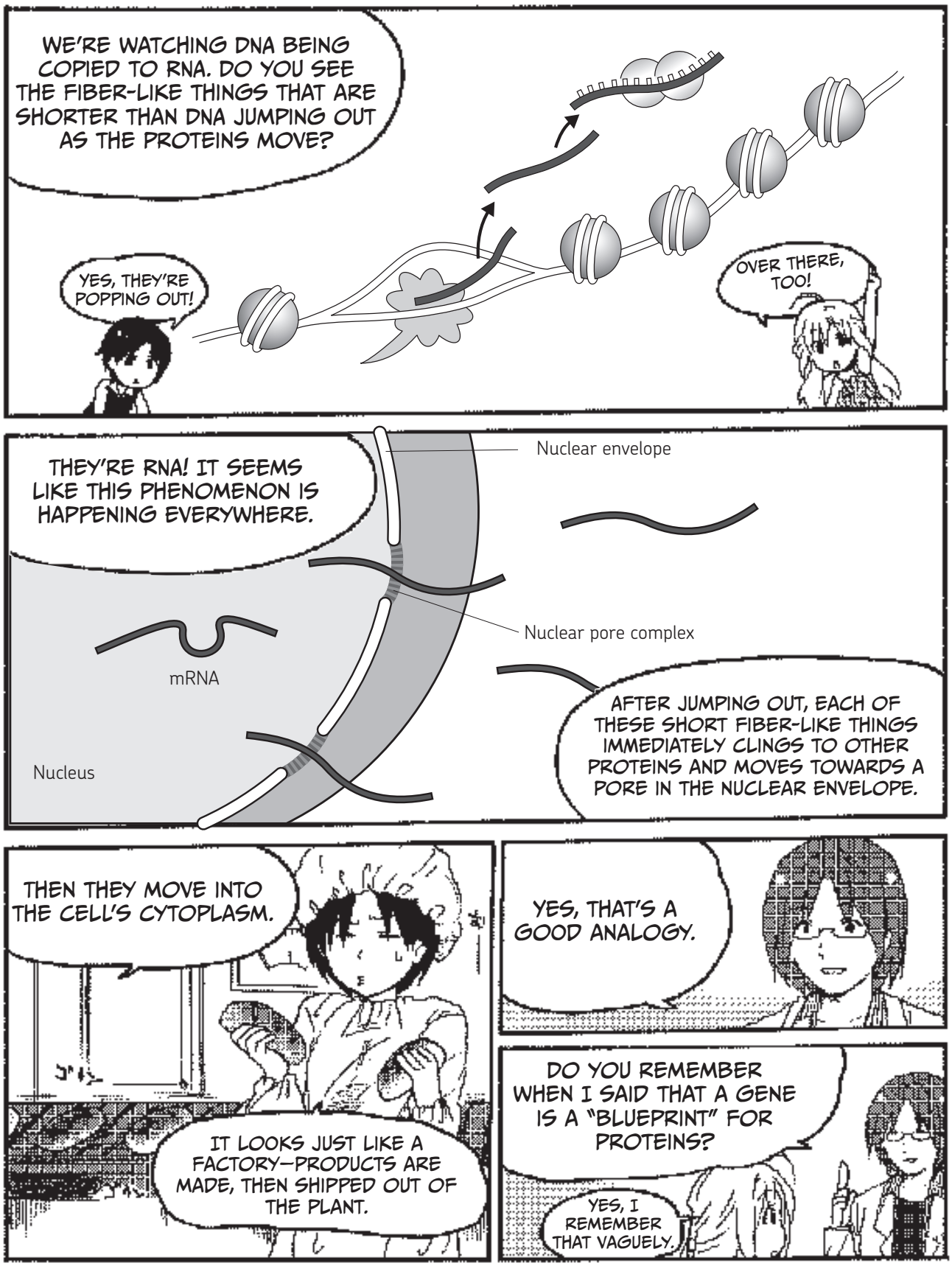

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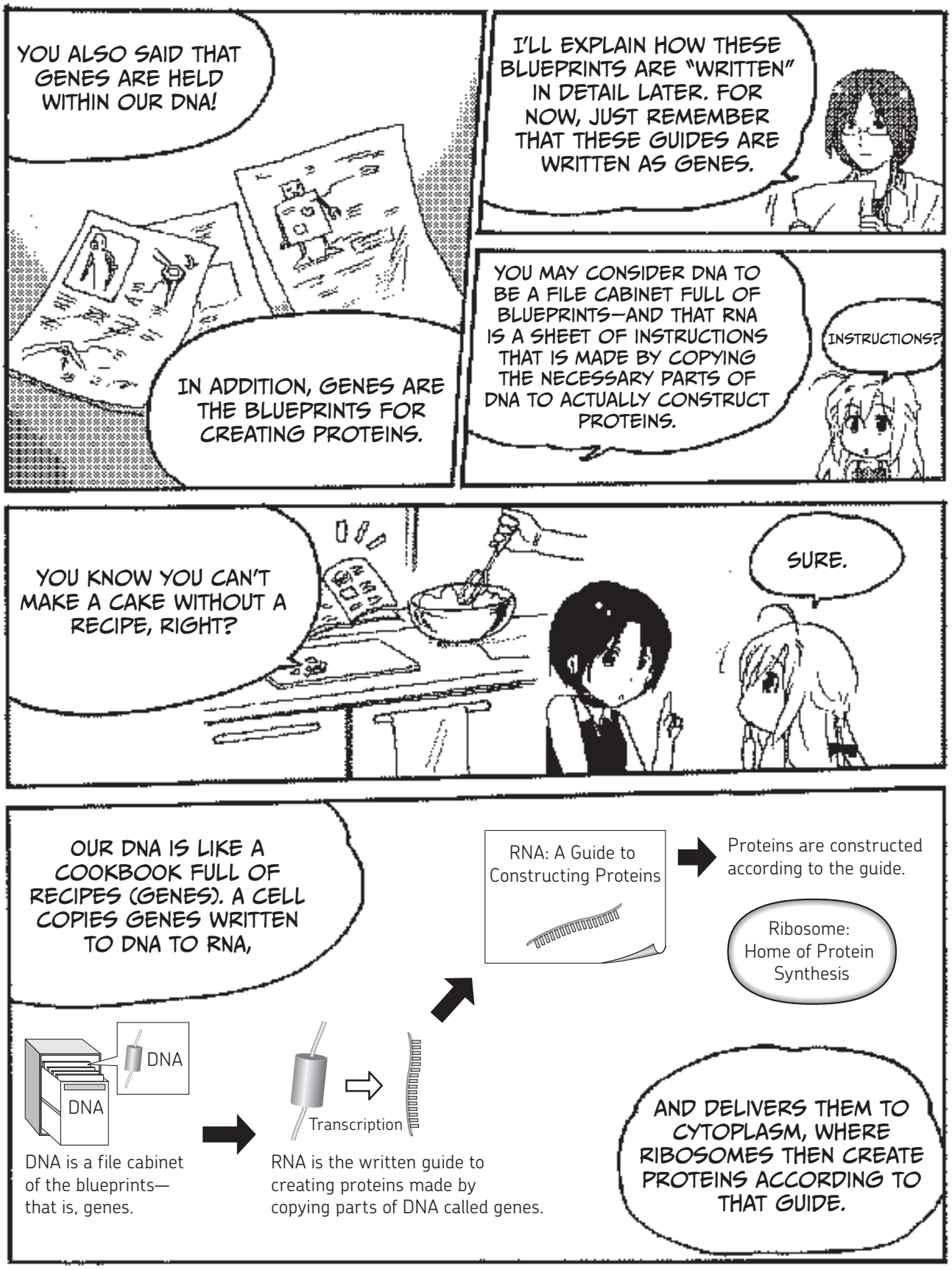

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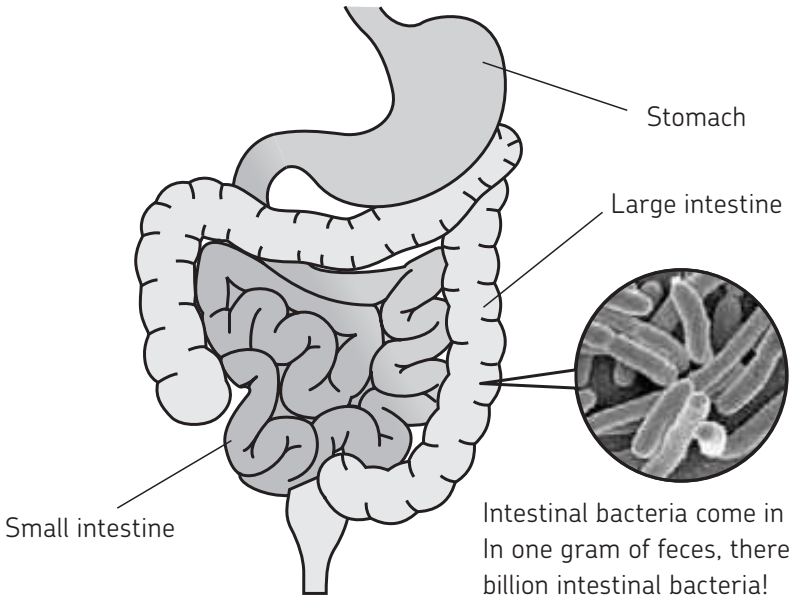
SINGLE-CELLED AND MULTICELLULAR ORGANISMS

I used the term *single cell* at the beginning of this chapter—the term can refer to one organism or to a "society" of living organisms.

A single-celled organism is a living creature made of only one cell. Most single-celled organisms are invisible to the human eye. You may think they are far away, but they're actually wriggling all around us.

In fact, we have trillions of single-celled organisms living inside our bodies. One example is intestinal bacteria, which live in our large intestines. Intestinal bacteria survive by grabbing nutrients from the debris of our digested food, but at the same time they prevent the propagation of harmful disease-causing bacteria. Our bodies and intestinal bacterial both benefit from each other in a "give and take" relationship. A mutually beneficial relationship between two organisms like this is called a *symbiotic* relationship.



Intestinal bacteria come in about 500 species. In one gram of feces, there can be up to one billion intestinal bacteria!

Bacteria are just one kind of single-celled organism. There are also protozoa—such as paramecia and amoebas.

Organisms with more than one cell are called . . . you guessed it, *multicellular*. Almost all living organisms visible to us (and many that aren't!) are multicellular: human beings, cherry trees, moss, dogs, fleas, and elephants are multicellular.

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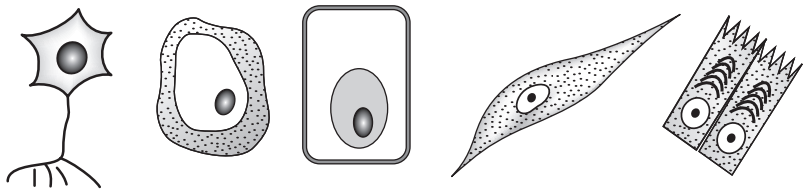
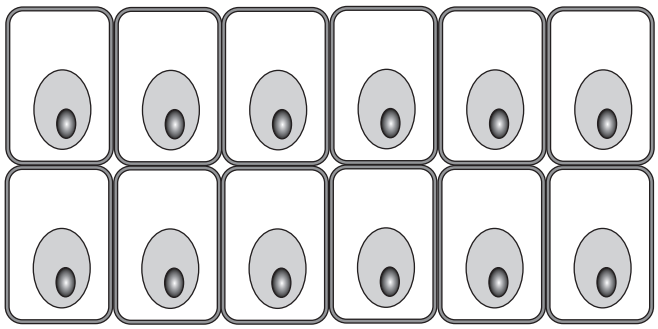


Various types of cells

Cells that make up different organs—nerves, stomach, and skin, for example—have different shapes and different functions. Cells with the same shape and the same function that gather together are called *tissue*. Bodies of animals, including human beings, are primarily made up of four kinds of tissue: epithelial tissue, connective tissue, muscular tissue, and nervous tissue.



Cells get together to form tissue.

Epithelial tissue (or the epithelium) This tissue forms the skin of organisms and the surface of internal organs, like digestive canals. There are several types, including flat epithelium, columnar epithelium, and sensory epithelium.

Connective tissue This tissue performs diverse roles throughout the body. It works to connect different tissues, cells, and organs to one another. Fibrous connective tissue, like ligaments and tendons, helps connect muscles to bone. Connective tissue is abundant in the protein collagen. Blood, bone, and adipose tissue (which you may know as fat!) are also types of connective tissue.

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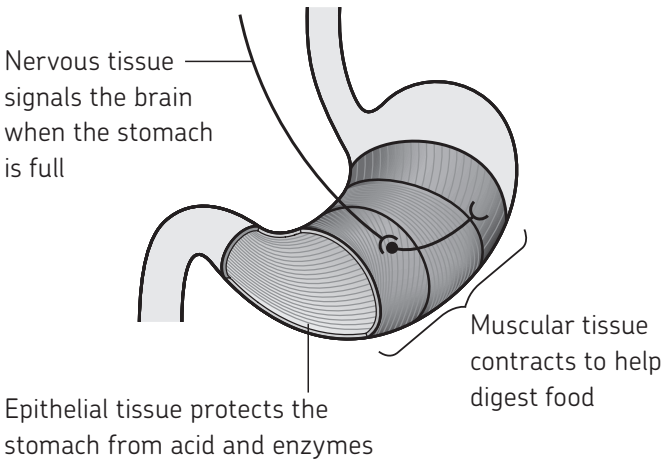
Muscular tissue As the name suggests, this tissue forms muscles. Skeletal muscle, cardiac muscle, and visceral muscle are included in this category.

Nervous tissue This tissue forms the nerves that make up the nervous system. Nerve cells use electrical signals to transmit messages back and forth from the brain to the rest of the body.



The tissues in your stomach: The connective tissue in the stomach is in a layer between the epithelium and muscular layer—it holds the whole stomach together.

A collection of tissues that are gathered together for a specific purpose is called an *organ*. Each tissue is joined to another in a specific way to form respective organs.

As you've seen, the stomach is made of a collection of four types of tissues.

Other organs are formed in a similar way, and carry out their specific duties. These organs in turn combine to form systems in our body.

Within multicellular organisms, cells gather together to form organs, and organs function together to form systems. In this way, a tiny cell can carry out very complicated tasks, indeed!

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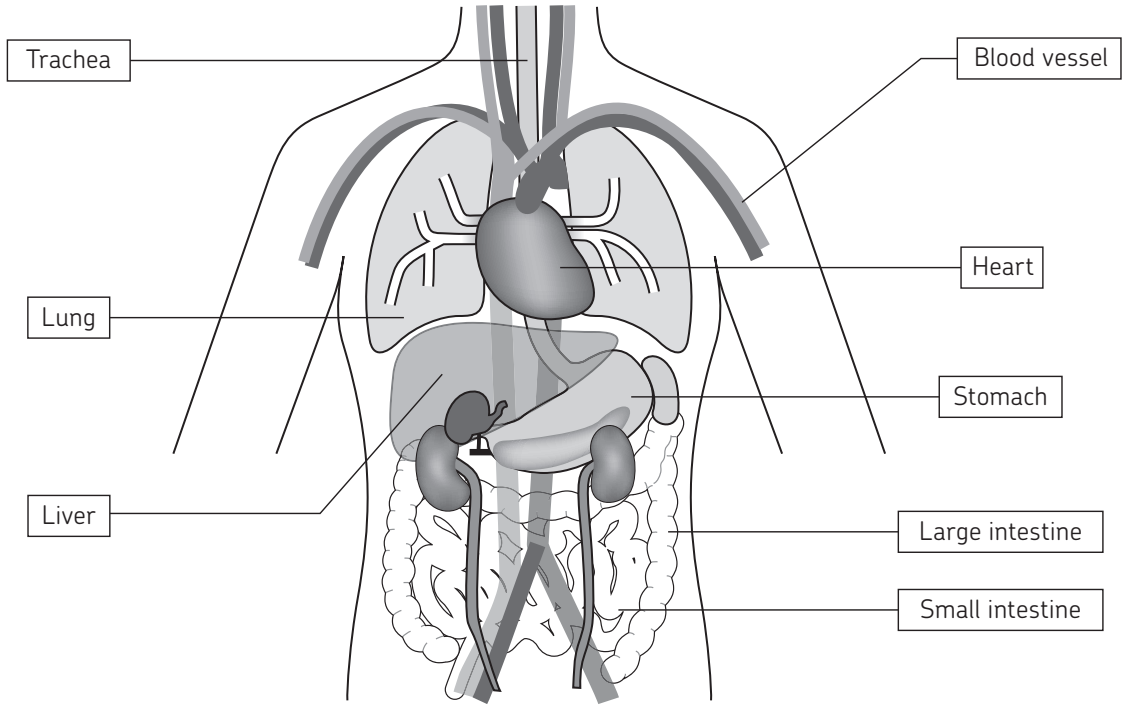

Organs in the digestive system: mouth, pharynx, esophagus, stomach, small intestine, large intestine, anus, rectum, liver, gallbladder, pancreas
Organs in the circulatory system: heart, aorta, arteries, veins, lymphatic vessels
Organs in the respiratory system: nares, pharynx, larynx, trachea, bronchi, lungs

PROKARYOTIC ORGANISMS AND EUKARYOTIC ORGANISMS

As you have learned before, the concepts of a single-celled organism and a multicellular organism are used for broadly classifying the world of living organisms into two groups. However, there is another way to classify the world of living organisms into two groups. This approach depends on the presence or absence of a nucleus in a cell. If this approach is employed, living organisms are broadly divided into prokaryotic organisms (those that don't have a nucleus) and eukaryotic organisms (those that do).

Presence or absence of a nucleus? Isn't the nucleus the "brain of the cell?" It's indispensable, isn't it?

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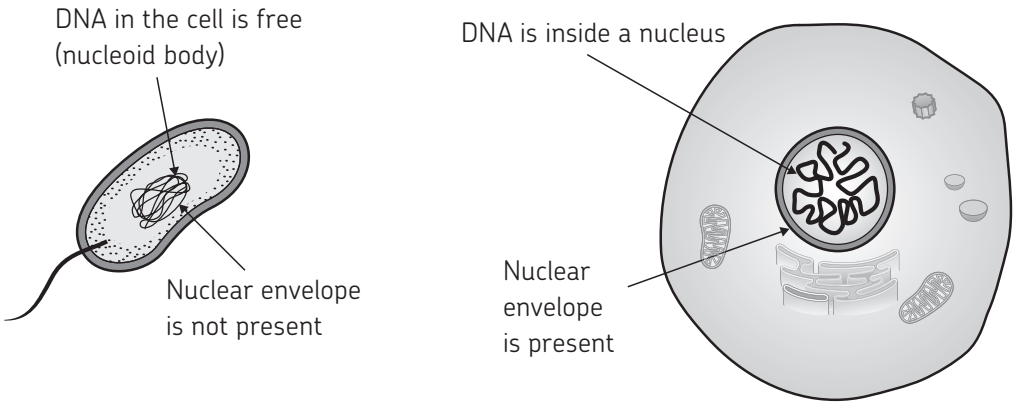
You might think so, but hold on for a moment. Try to remember why the nucleus is called the "brain." It's because the nucleus stores DNA and controls the expression of genes written in DNA, right? But as long as the DNA is in the cell and the genes are appropriately expressed, does the structure called the nucleus matter? When the genetic material of an organism is not held within a nucleus, this organism is called a *prokaryotic organism*. Bacteria are the only prokaryotic organisms on the earth. In prokaryotic organisms, all important jobs of the cell are done within the cytoplasm, instead of within membrane-bound organelles.



Prokaryotic organism

Eukaryotic organism

When the nucleus of an organism has a membrane and a distinct shape, this organism is called a *eukaryotic organism*. Every single-celled organism (excluding bacteria) is included in the eukaryotic category. Protozoa such as paramecia are eukaryotic organisms.

You might think the terms *non nucleated organism* and *nucleated organism* would be sufficient for classifying organisms, depending on the presence or absence of a nucleus. But that is not correct. Although we say that prokaryotic organisms do not have nuclei, they actually do have their own structure that functions the same as a nucleus. The area where DNA is stored (it's contained in a nucleoid body) is separate from the surrounding cytosol. Thus, the term *prokaryon* is used to indicate the existence of a nucleuslike structure (like the nucleoid body), more primitive than a true nucleus. This prokaryon is not surrounded by a nuclear envelope like a true nucleus.

This term *true nucleus* is used for organisms with a membrane surrounding and protecting their genetic material. The nuclear envelope is just a membrane, but it's an important one!

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2

PROTEINS AND DNA: DECIPHERING THE GENETIC CODE

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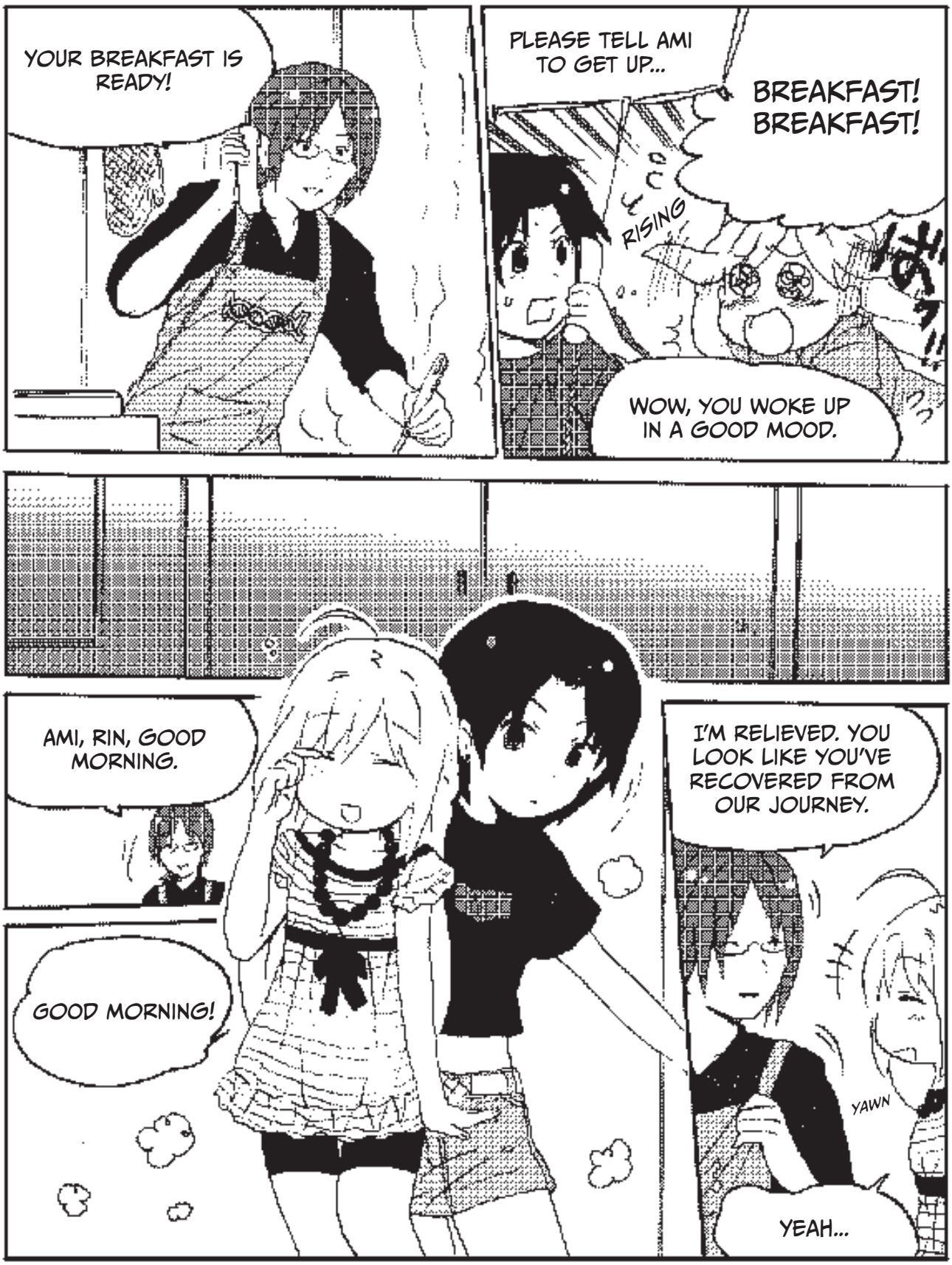

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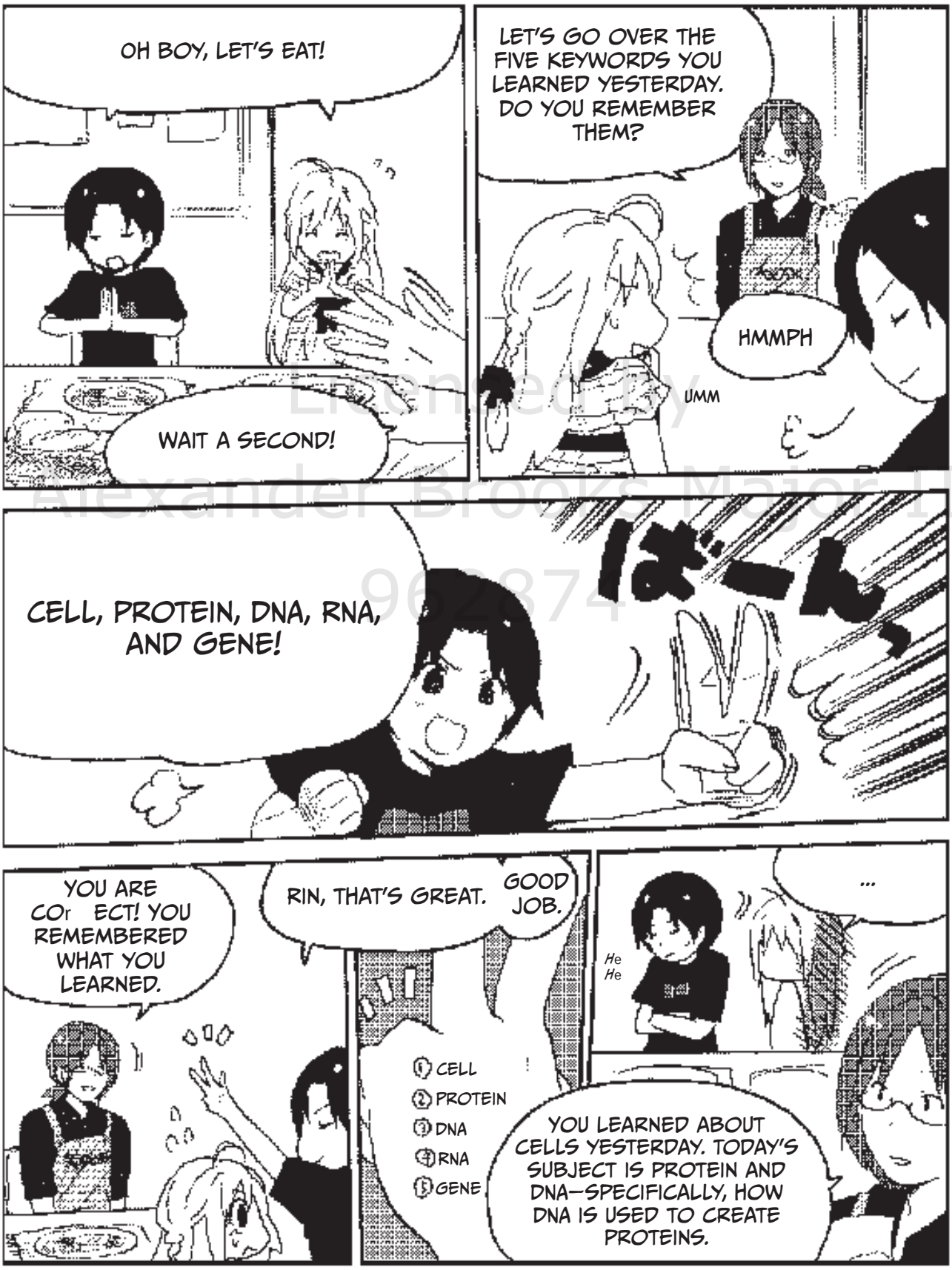

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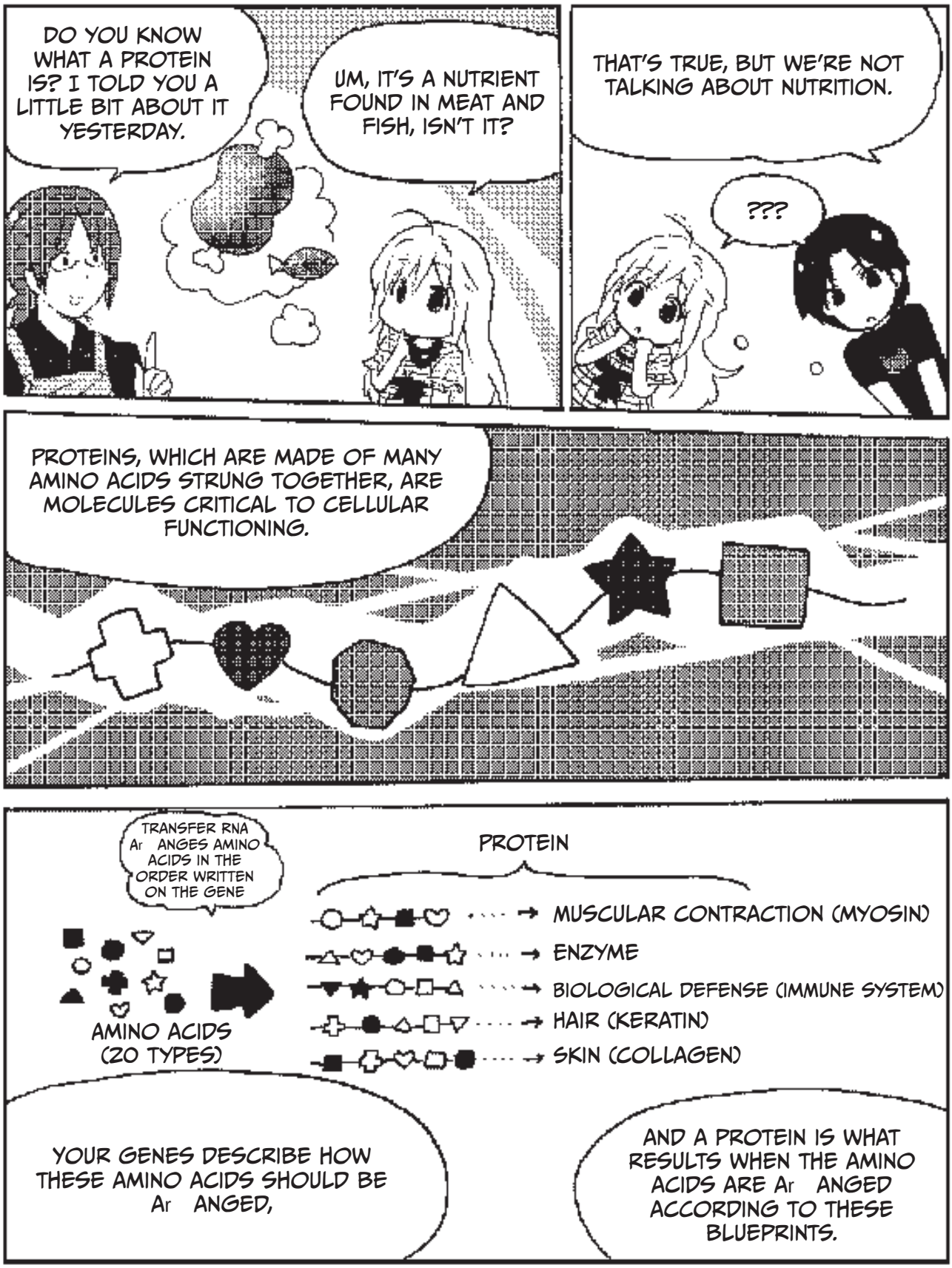

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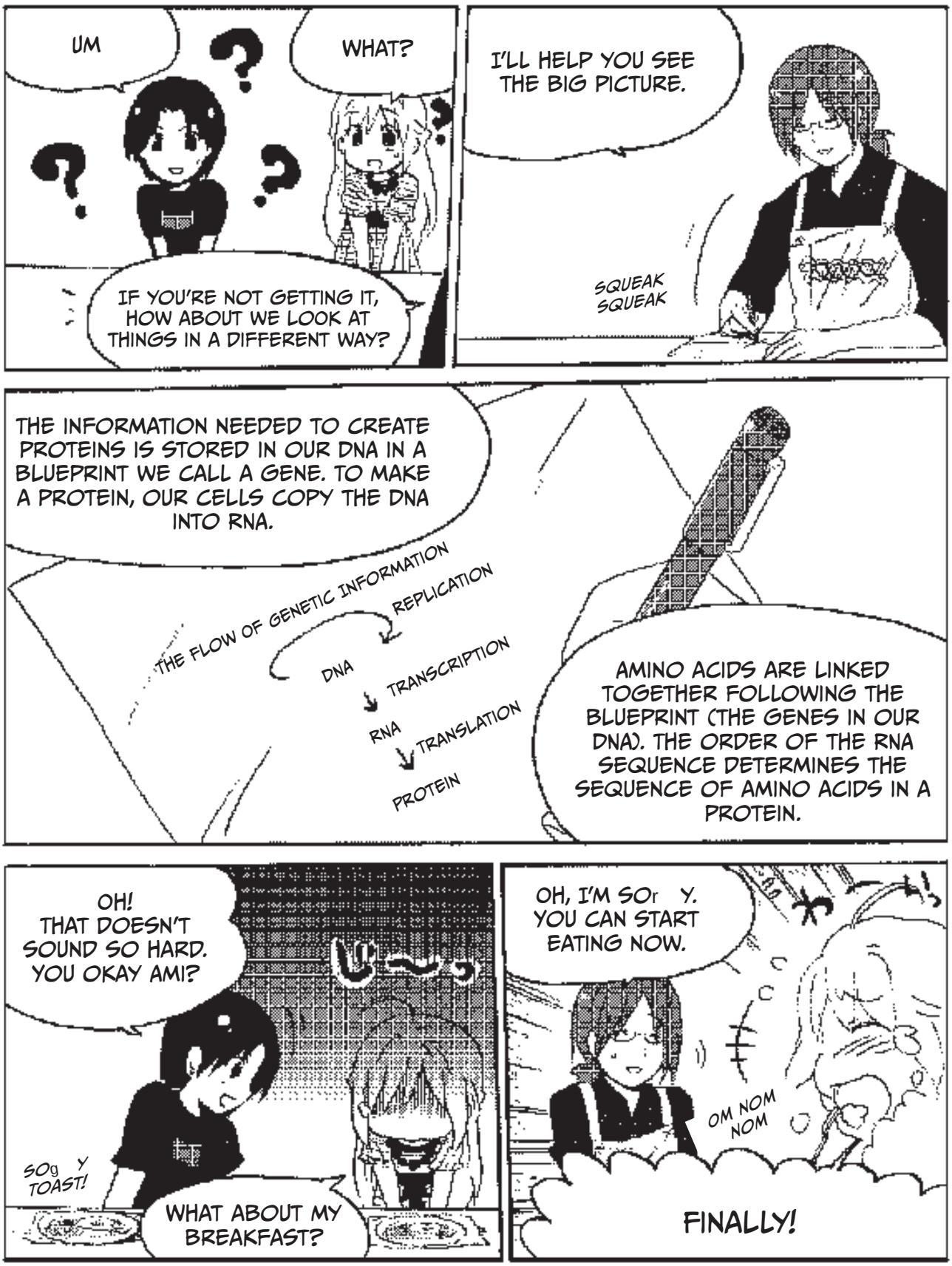

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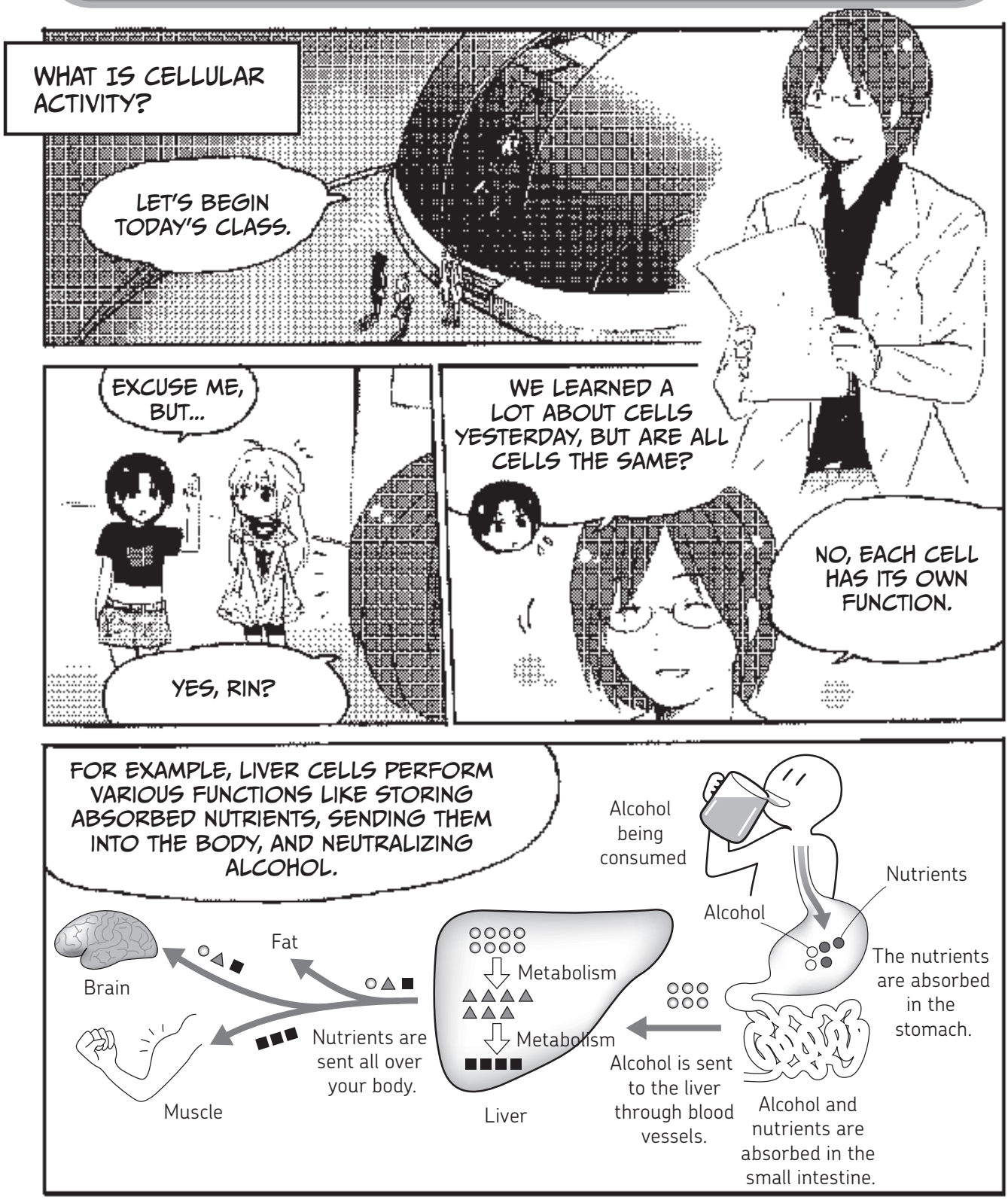
PROTEINS DRIVE CELLULAR ACTIVITY



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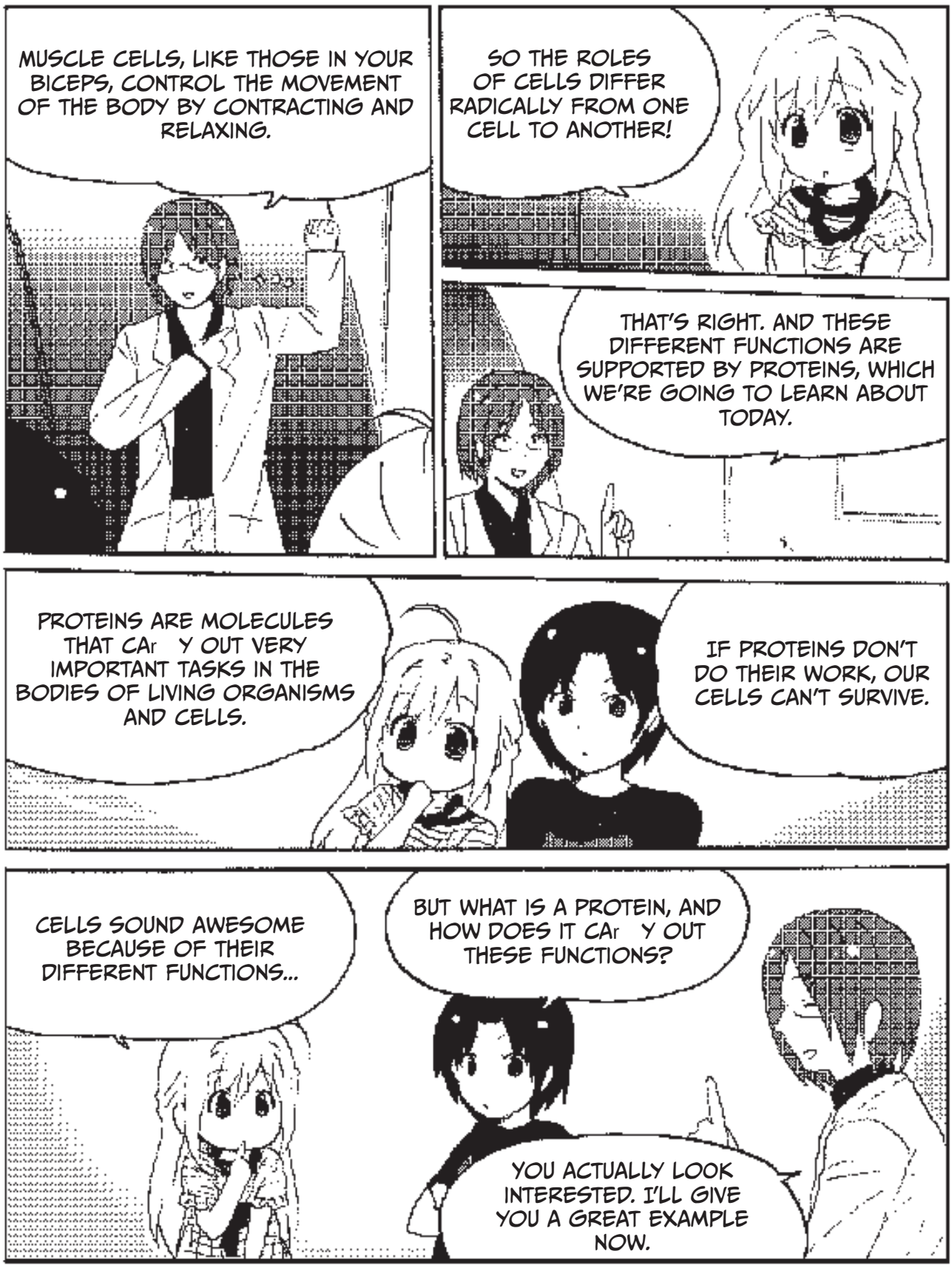

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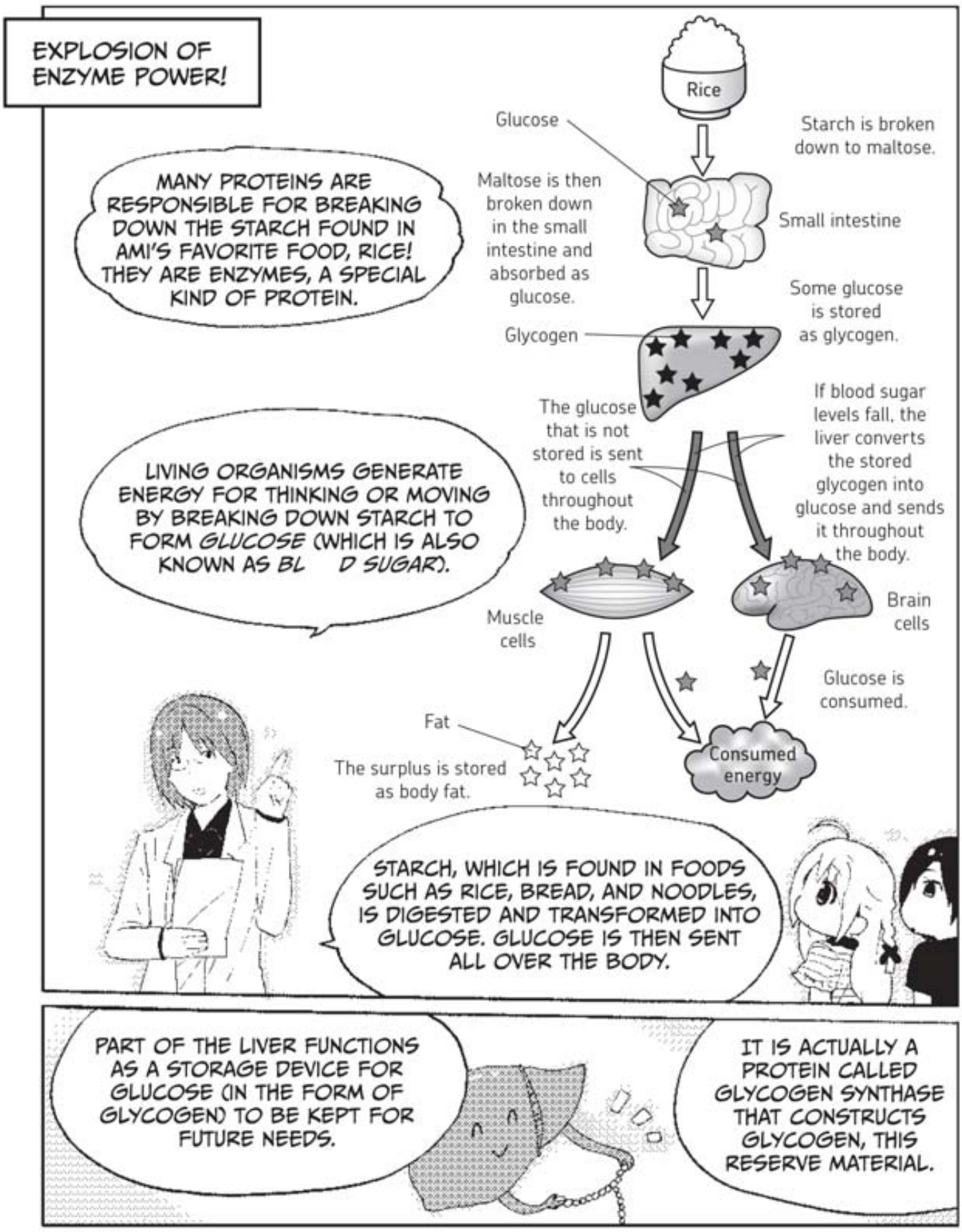

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* ALTHOUGH ALMOST ALL CELLS OF LIVING ORGANISMS STORE GLYCOGEN, MOST OF IT IS STORED IN THE LIVER AND MUSCLES.

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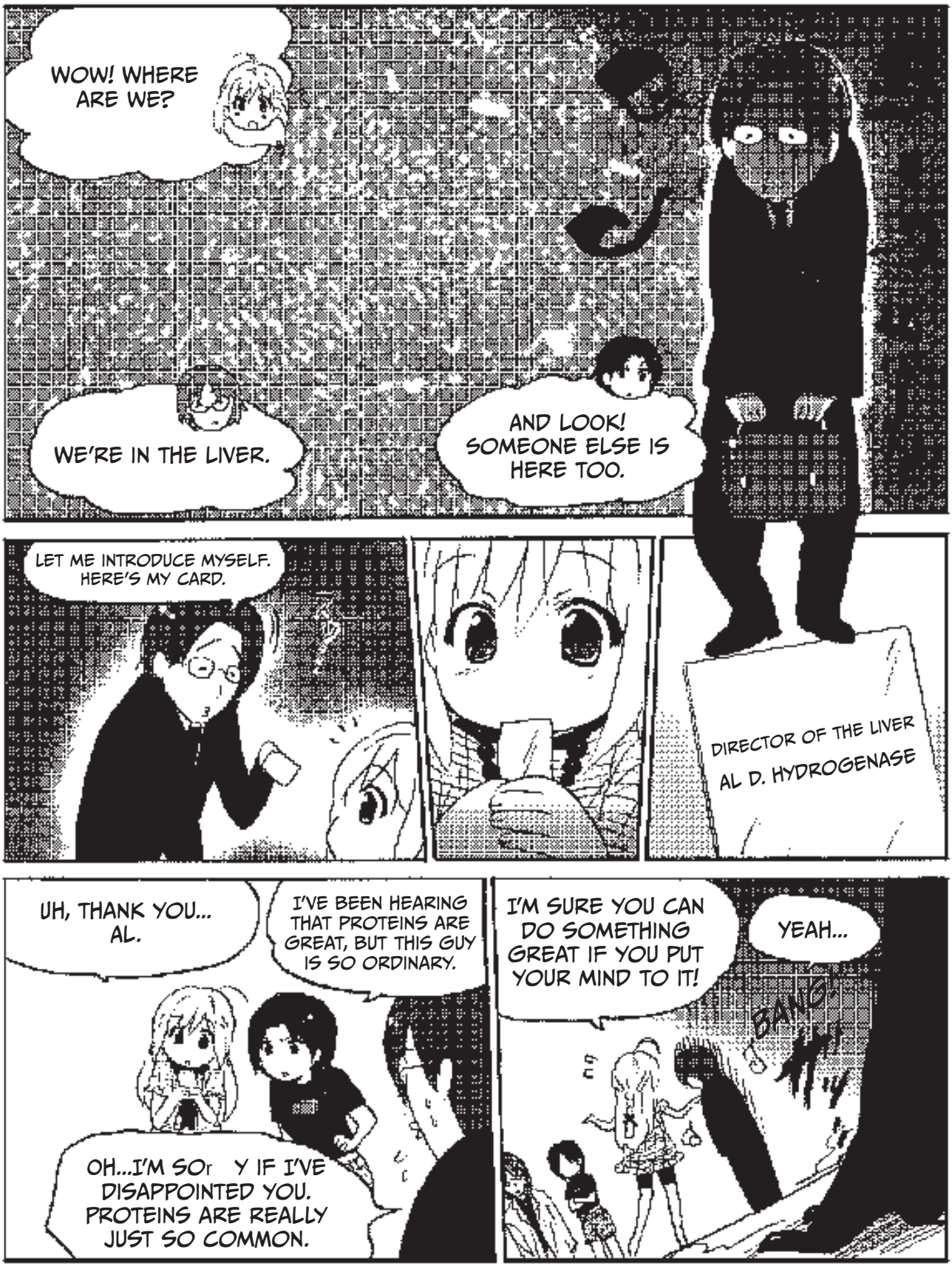

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* DRINKZILLA REPRESENTS ALCOHOL IN THIS SCENARIO.

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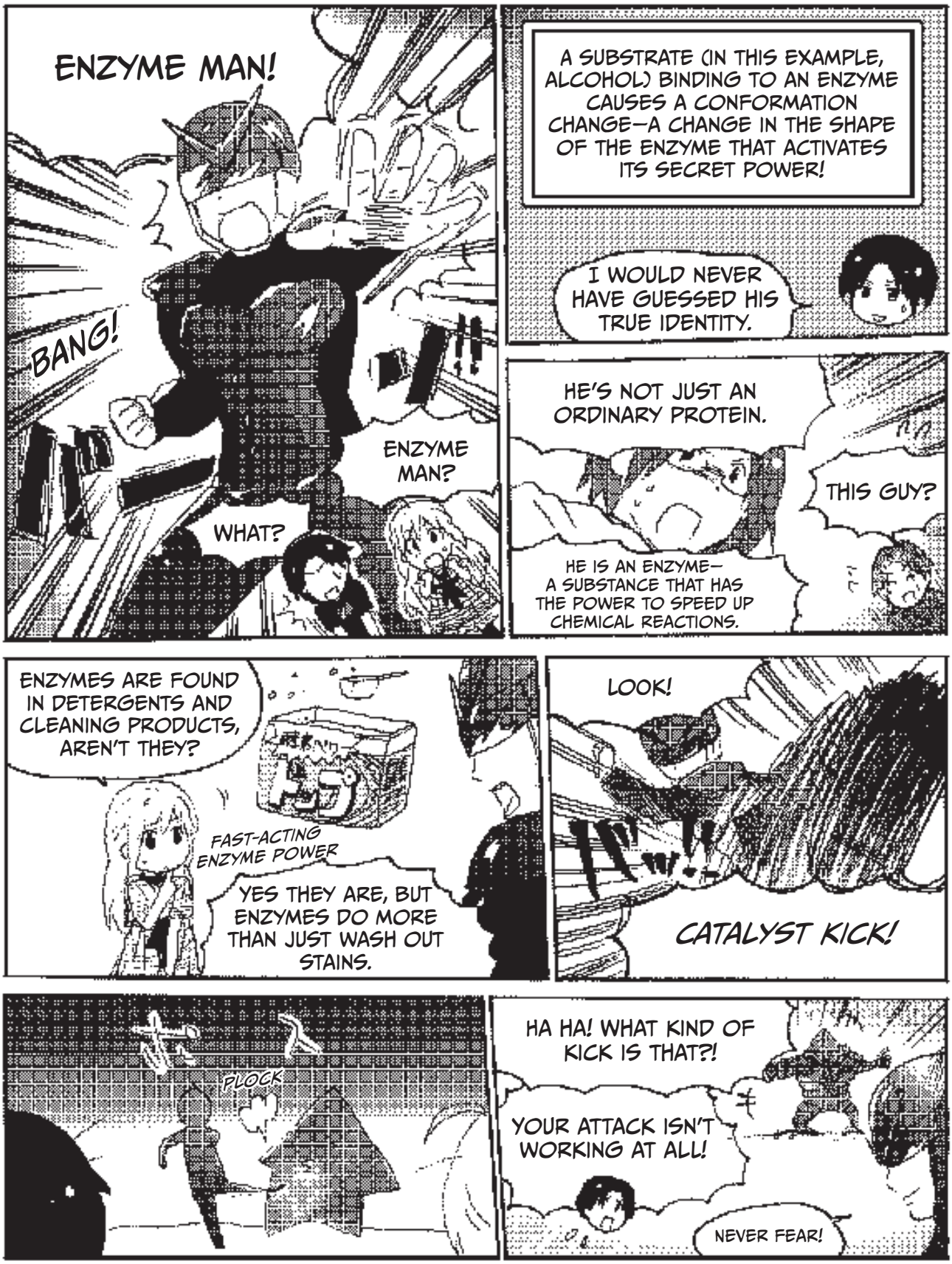

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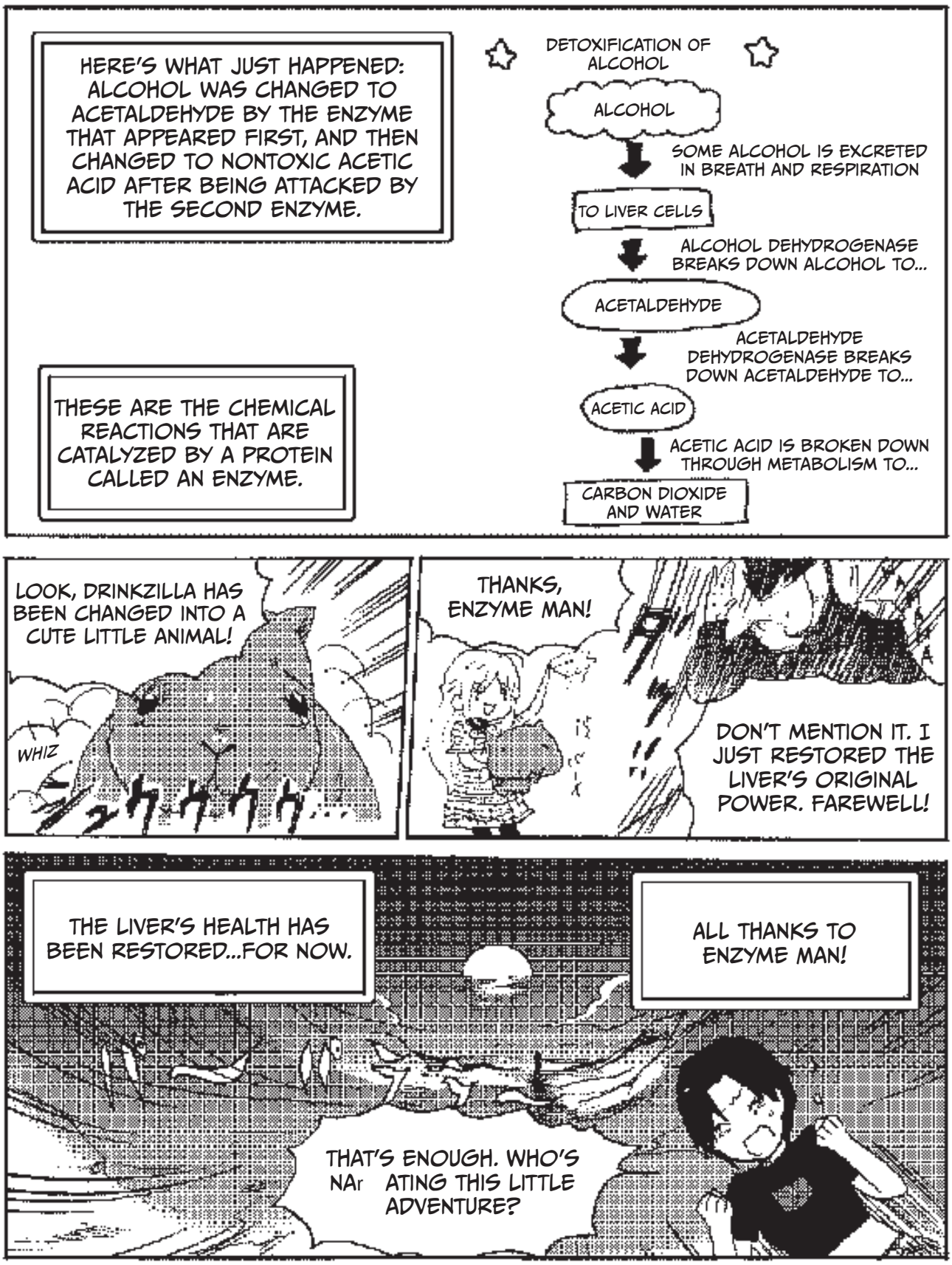

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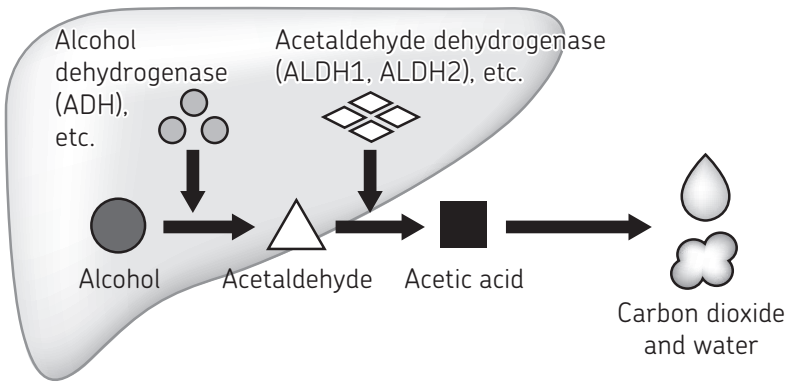
PROTEINS ACTING AS ENZYMES

Enzymes are a special type of protein that have the power to accelerate chemical reactions. There are tens of thousands of types of proteins in our bodies, but not all of them are enzymes. You'll soon learn that some proteins, such as those that fight bacteria as part of our immune system, are not enzymes.

Various processes that happen within our bodies, such as digestion, absorption of nutrients, and replication of DNA, occur due to chemical reactions. A different protein is responsible for each chemical reaction. A unique enzyme starts almost all the chemical reactions carried out by living organisms. All enzymes are *catalysts* (or *biocatalysts*). Catalysts help start chemical reactions and make them easier to move forward, but they do not actually react with any other molecules in the reaction.

That's why Enzyme Man called his attack a *Catalyst Kick*!

By the way, the proteins we saw that stored glucose and detoxified alcohol are all enzymes. The protein that stores glucose as glycogen is called *glycogen synthase*, and the protein that degrades alcohol to the harmless acetaldehyde is *alcohol dehydrogenase*.



As we saw earlier, alcohol is broken down in the liver. One enzyme breaks down alcohol to acetaldehyde, and yet another enzyme further breaks down acetaldehyde into acetic acid.

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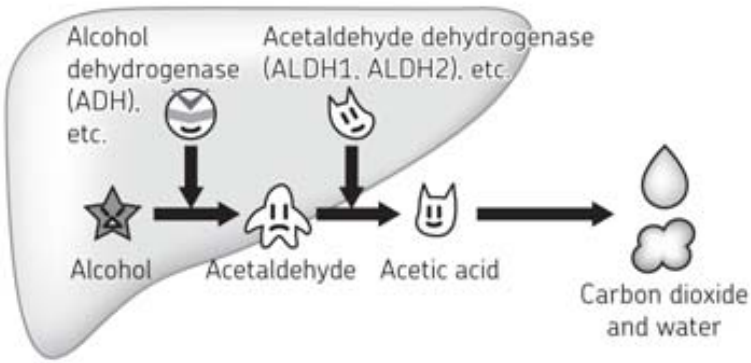
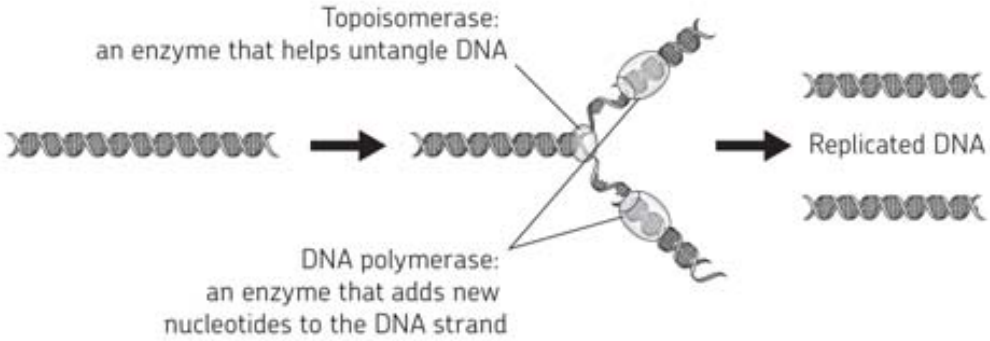
Do you remember? It was Enzyme Man, or alcohol dehydrogenase, that broke down alcohol, and his sidekick dog, acetaldehyde dehydrogenase, that broke down acetaldehyde to acetic acid. A different enzyme is responsible for each chemical reaction.



PROTEINS' ROLE IN CELL DIVISION

Proteins also run the process of cell division. Division is how a cell reproduces, that is, by splitting in two. Before a cell undergoes division, the DNA in the nucleus is copied so that one copy may be given to the two new cells formed after division. An enzyme is responsible for starting this process of copying DNA (called *DNA replication*).



When a cell undergoes division, enzymes are not the only proteins that are working. The structural proteins that give the cell its shape also help move the contents of the cell and the cell membrane in preparation for cell division.

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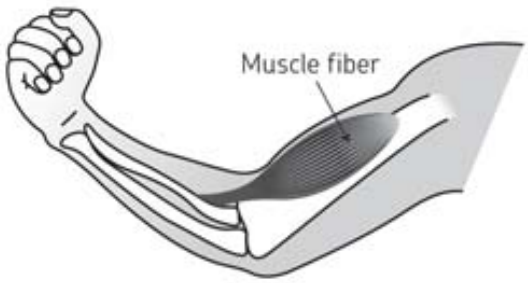
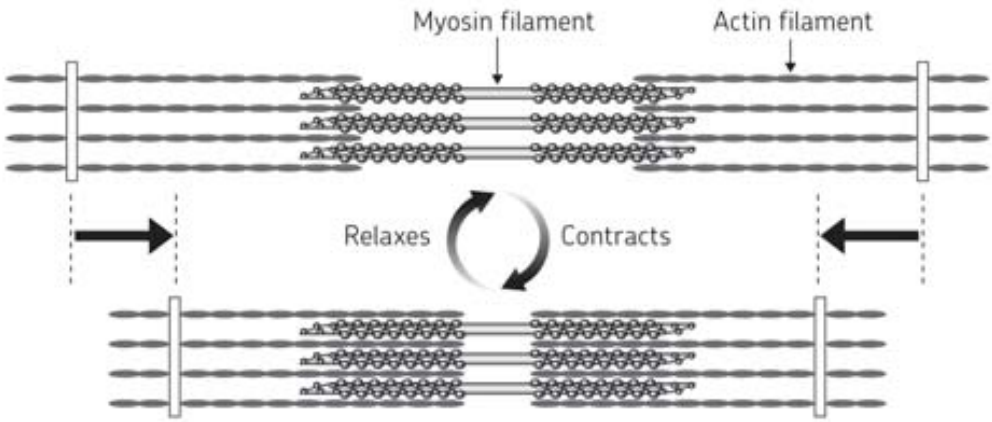
PROTEINS AND MUSCLE CONTRACTION

Marcus, are there proteins in muscle cells, like the biceps you talked about at breakfast?

Good question. Muscles like biceps are formed by a group of bundles of muscle cells. Each cell is called a *muscle fiber*.



Muscle cells are made up of two long, fine fibers called *actin filament* and *myosin filament*. These fibers are made up of two types of proteins called *actin* and *myosin*, respectively. A muscle contracts when the fibers slide against each other.



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Proteins help muscles maintain their shape, and they move muscles themselves. They're not simply catalysts for a chemical reaction.

I guess Enzyme Man had a bit part after all.

Rin! That's not very nice. I feel sorry for Enzyme Man.

Oh, whatever. He definitely didn't do as much as a protein in a muscle does.

SUMMARY

Proteins help cells perform various functions. Over 100,000 types of proteins exist in the human body. Each protein is responsible for carrying out specific work.

These are some of the main functions of proteins:

- Controlling chemical reactions (enzymes)
- Contracting muscles (actin and myosin)
- Transporting oxygen and nutrients (hemoglobin)
- Maintaining the homeostasis (hormones like insulin)
- Defending the body from viruses and harmful bacteria (immunoglobin)
- Maintaining the structure of cells (collagen and keratin)

Proteins perform vital functions in the body.

I get it, proteins are great.

Yes, we can carry on living thanks to the ongoing work of proteins.

Oooh, proteins like Enzyme Man! He's so dreamy.

What's with you and Enzyme Man?!

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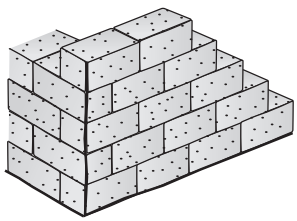
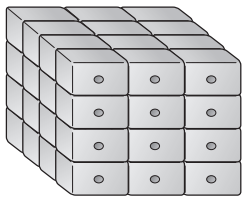
PROTEINS ARE MADE OF AMINO ACIDS

We learned yesterday that living organisms are made up of cells. Groups of cells form tissues, and groups of tissues form organs, which ultimately form the body of living organisms. It's like stacking up bricks to build a house.



Stacked bricks make a wall.



Cells join together to form tissue.

Proteins also consist of smaller components—specifically, chains of molecules called *amino acids*.

Proteins are made of chains of amino acids.

DNA is also made up of chains—the substances that link together to form DNA are called *nucleotides*.

DNA is made of a chain of nucleotides.

Do amino acids form proteins the way cells form tissues?

No! There is a big difference between cells and proteins. Cells pile up in three dimensions to form body tissues, but amino acids link horizontally to form proteins.

So if cells are like bricks, amino acids are like beads on a necklace, right?

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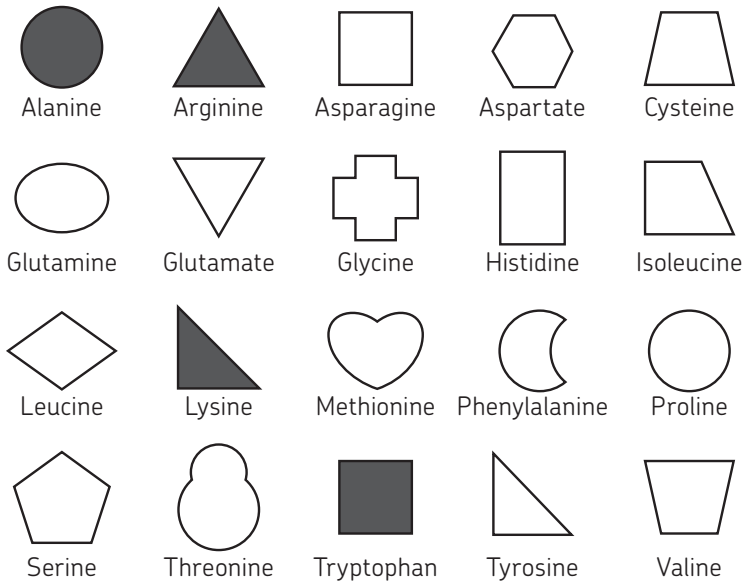
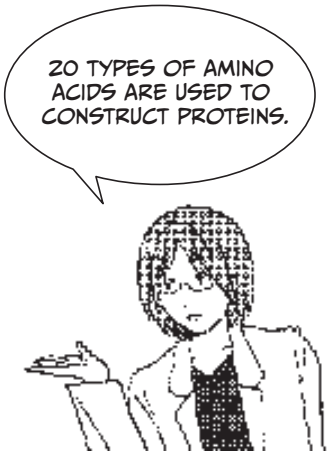
Exactly. Of course, these strings take unique jumbled and tangled shapes as well. They certainly aren't just in a straight line.

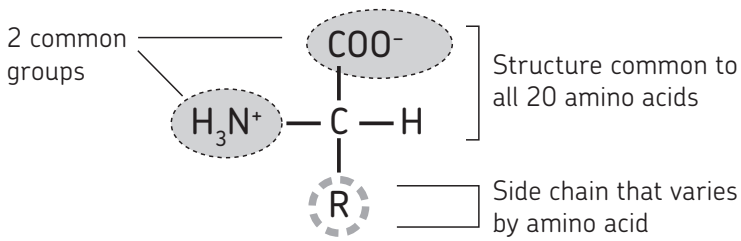
These amino acids link together to form proteins and their sequence determines their function.





MSG is made up of an amino acid, right? It makes my food taste so good!

Yes, that's right. MSG is made of glutamic acid, one type of amino acid. There are 20 types of amino acids, and arranging them in a predetermined order creates a specific protein. (There are actually more than 20 types of amino acids, but this is how many are used for creating proteins.) Let's look at the structure of amino acids. Each of the 20 amino acids used for creating proteins has a portion common to the other 19 amino acids, as well as a unique portion not found in the others.



In this figure, *R* represents a side chain: This portion varies in each amino acid. So there are 20 types of side chains, ranging from simple ones

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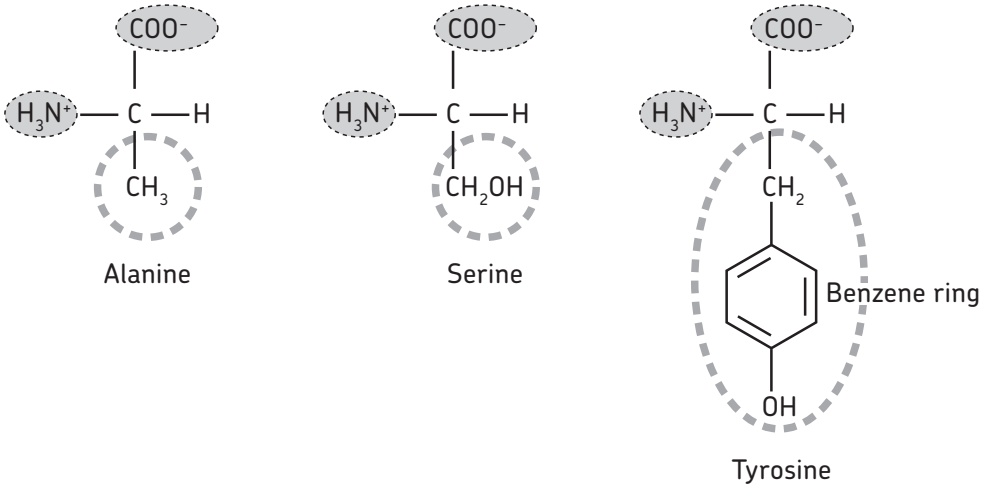
consisting of a single hydrogen atom to complex ones made up of several linked benzene rings.



Each of these 20 amino acids shares a common structure of H_3N^+ and COO^-.

REPLACING ONE AMINO ACID WITH ANOTHER IS A BIG DEAL!

This may seem unrelated, but do you know why human blood is red?

Because human blood is red with the flame of justice—just like Enzyme Man burns with passion for doing good!

Uh, that can't be right.

No, indeed, it's not. The correct answer is because of the red pigment called hemoglobin found in blood. Red blood cells carry out the important function of bringing oxygen molecules to cells throughout the body by attaching these oxygen molecules to hemoglobin. Once they have oxygen, the cells can generate energy. Hemoglobin also carries CO_2. Hemoglobin is bright red because of the high quantity of iron contained within it.

But Marcus, why are you suddenly talking about blood? What about proteins?

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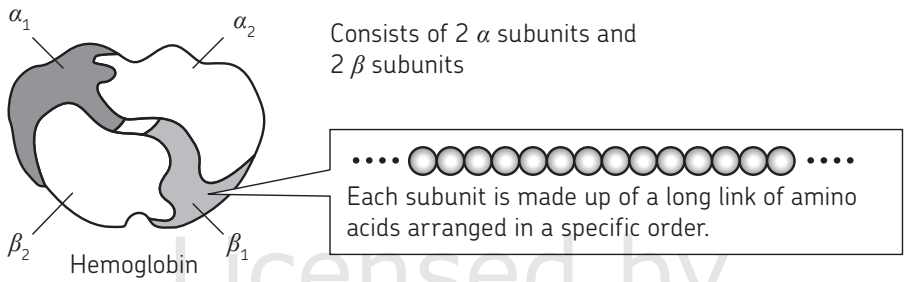
Well, the main component of hemoglobin is protein! Hemoglobin is made up of two types of proteins called globins: α and β. Each of these proteins is called a *subunit*. There are two α subunits and two β subunits. Because they are proteins, each of the two types of subunits is made up of a long sequence of 20 types of amino acids linked together in a particular order.



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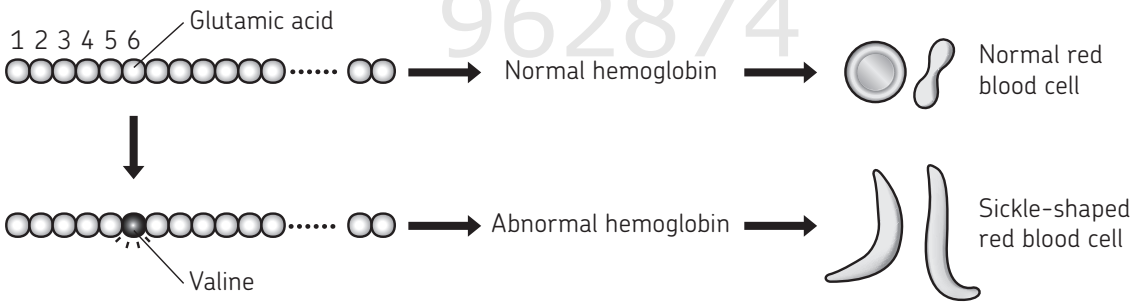
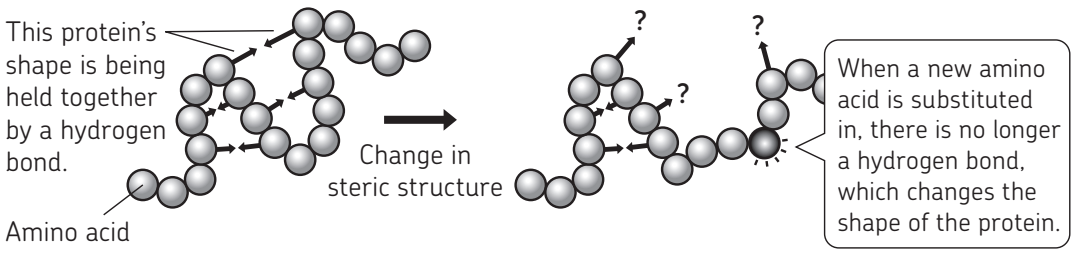
Changing just a single amino acid in the chain can cause serious trouble. If, for instance, the sixth amino acid contained in the β subunit, glutamic acid, is replaced with valine, an abnormal deformation occurs in hemoglobin. This deformation prevents the hemoglobin from carrying oxygen properly and causes anemia. Switching amino acids can also cause red blood cells to deform into a sickle-like shape, causing sickle-cell disease.



The order amino acids are arranged in is also important. Each unique *R* portion of amino acid pulls every other *R* portion with a particular force, such as electrostatic force, the force of a hydrogen bond, and the force of hydrophobic interaction. The forces between R groups force the molecule to bend into a three-dimensional shape. The structure of an entire protein (the *steric structure*) is determined by the balance of these forces. Replacing a single amino acid with another results in a change in the shape of the protein. And for proteins, shape and function are one and the same!



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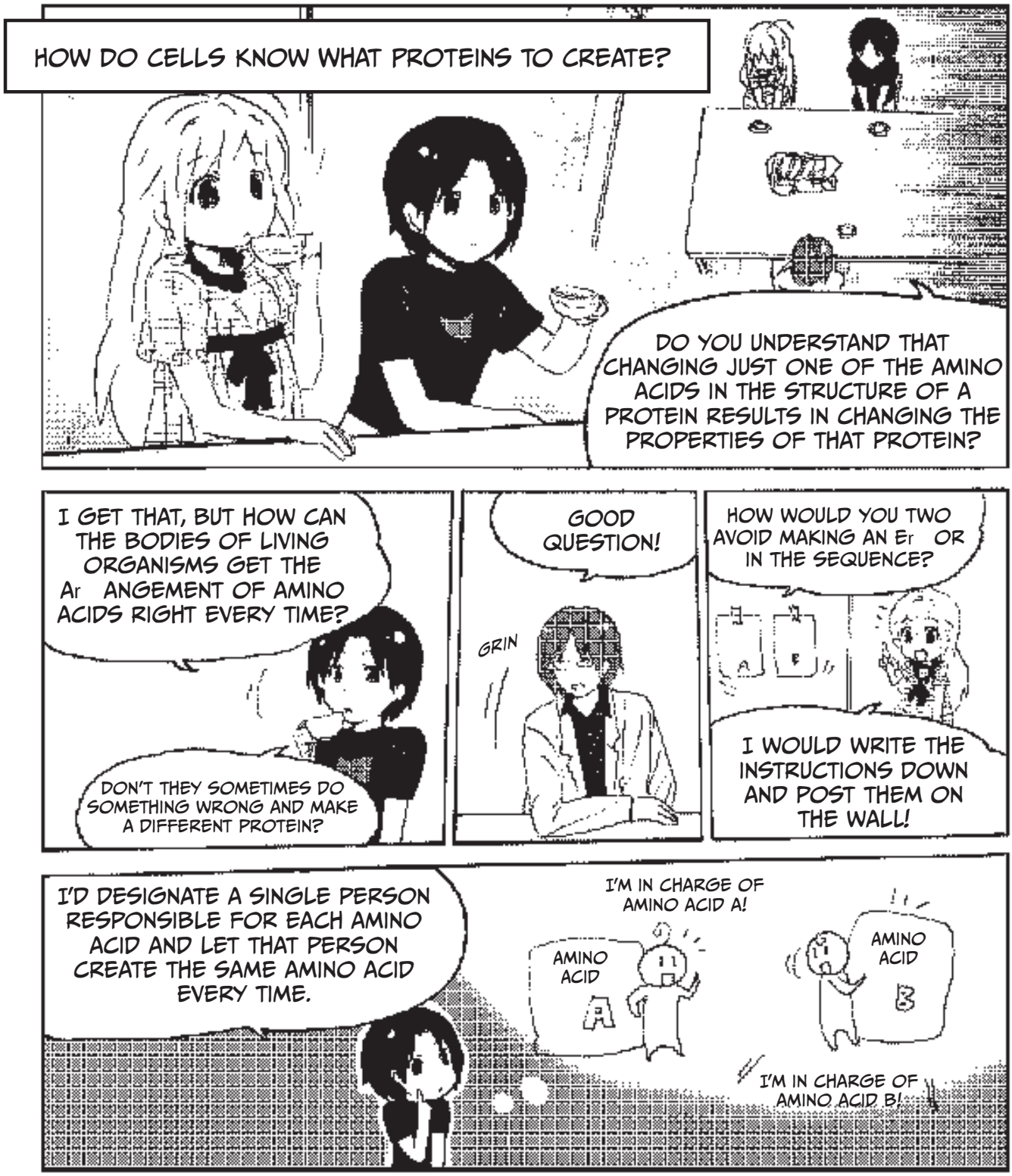

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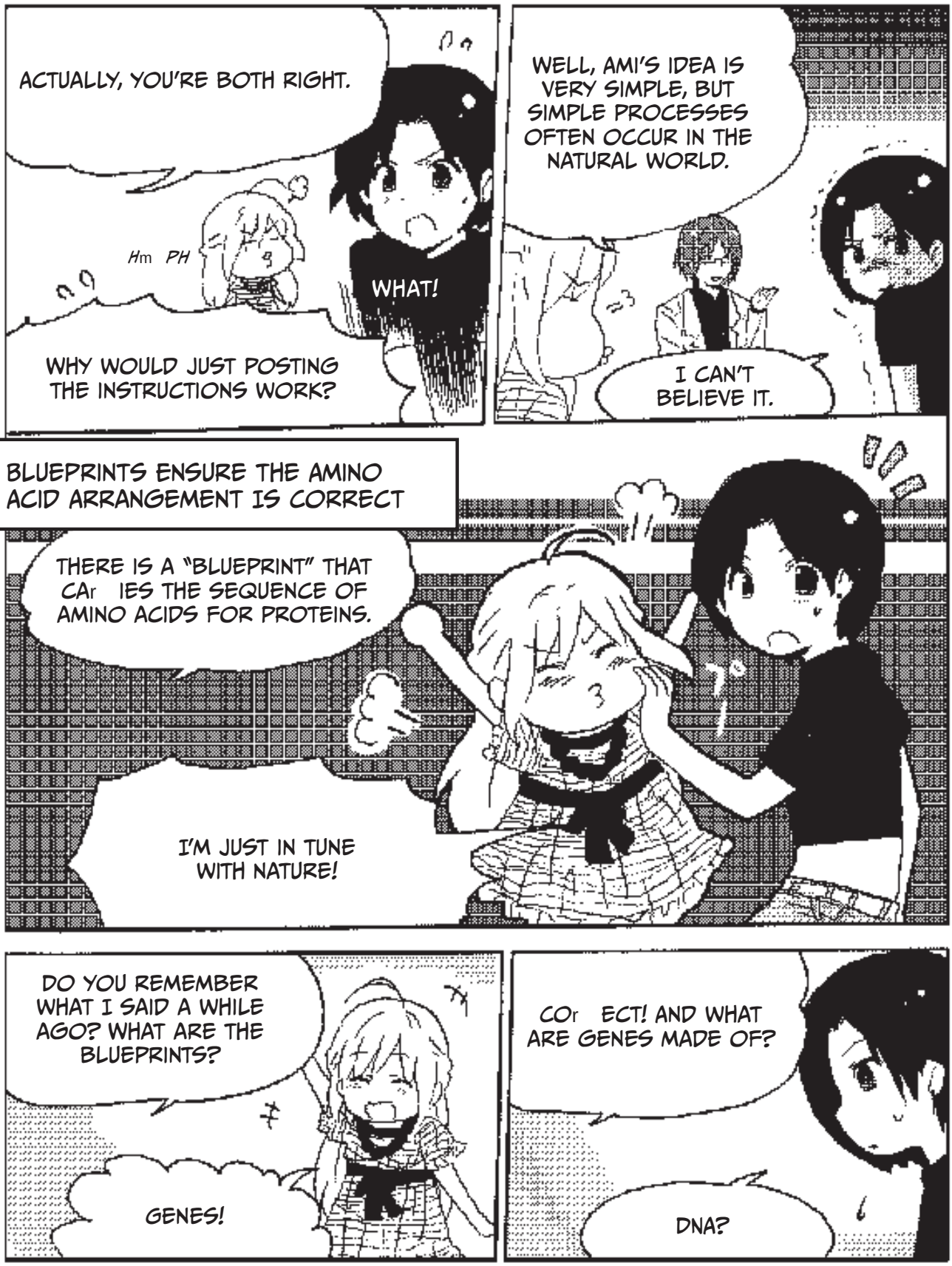

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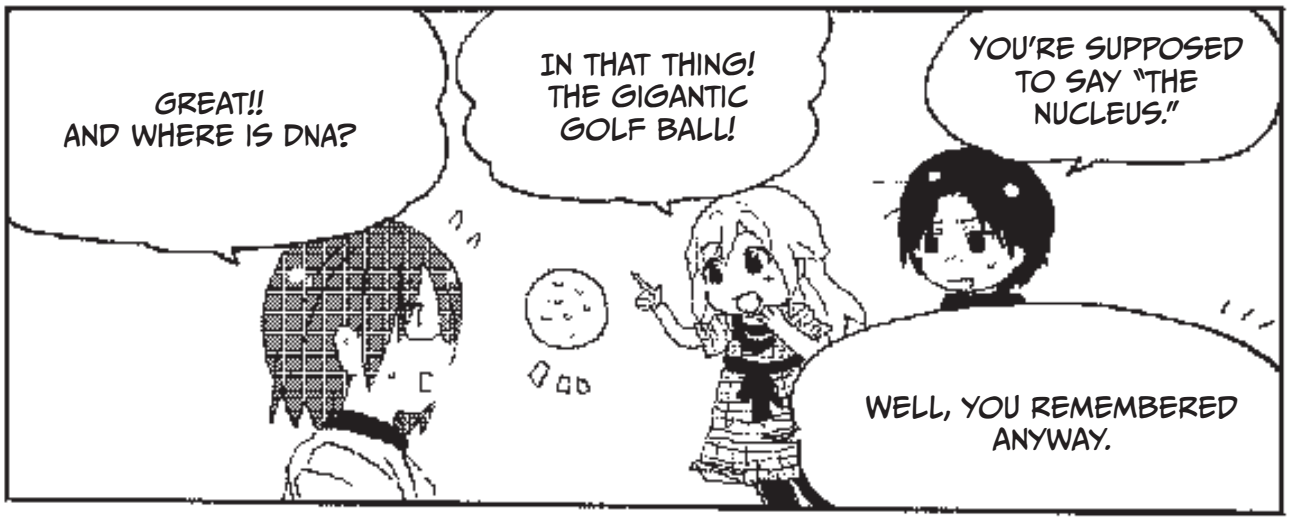

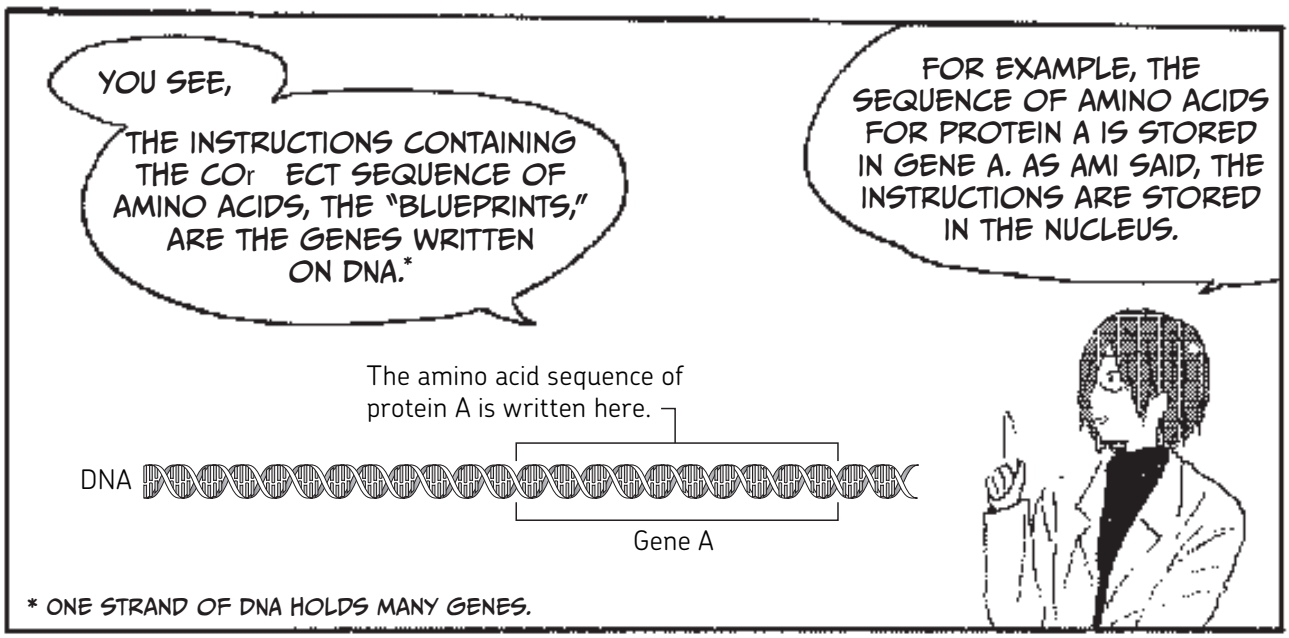

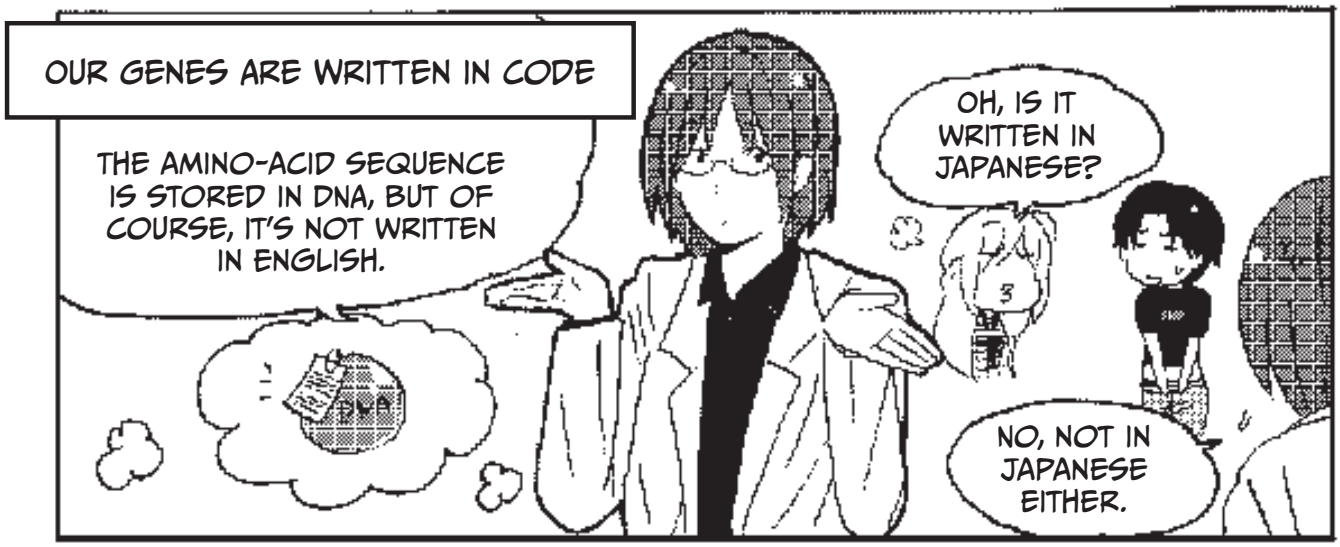

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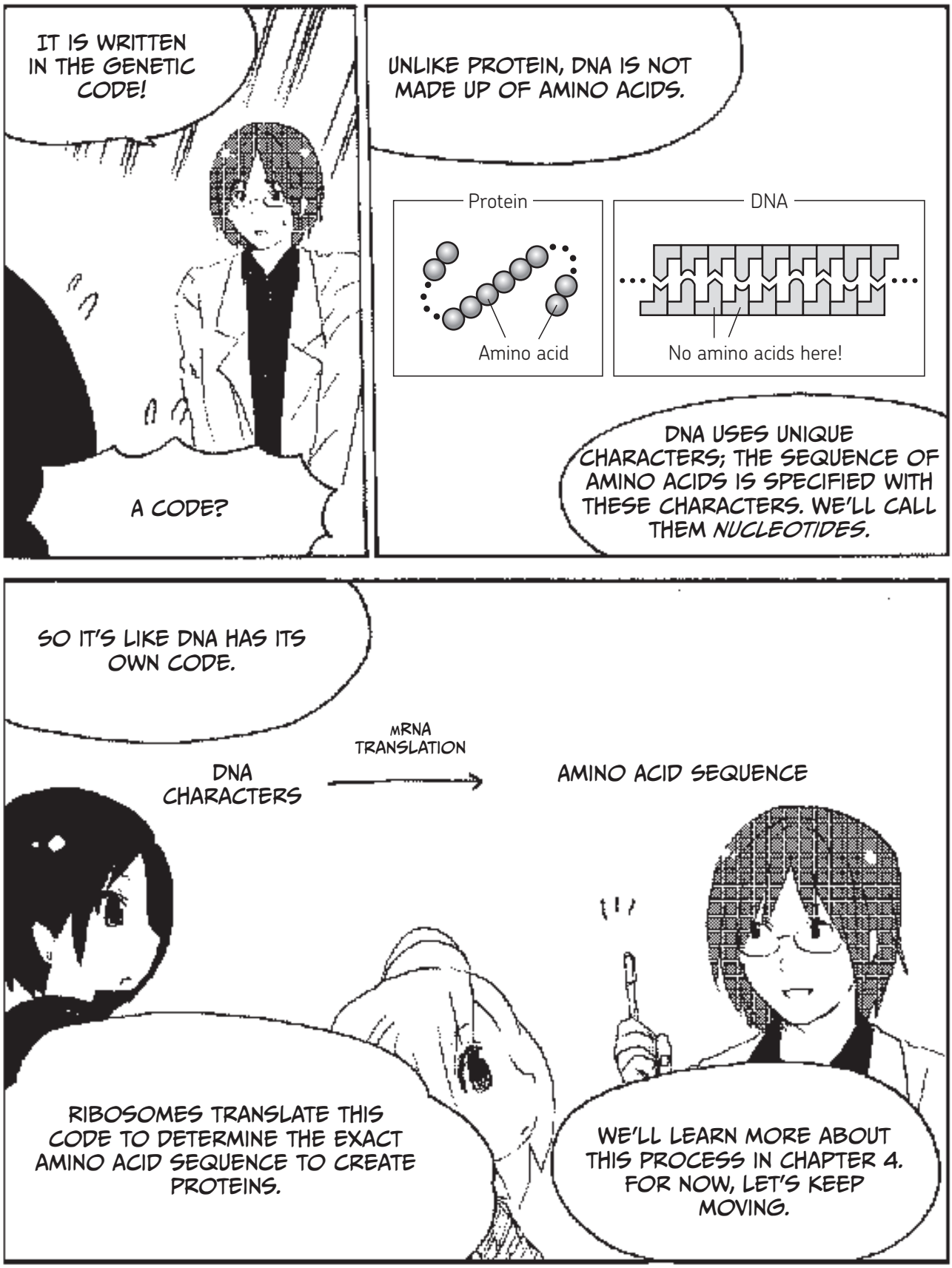

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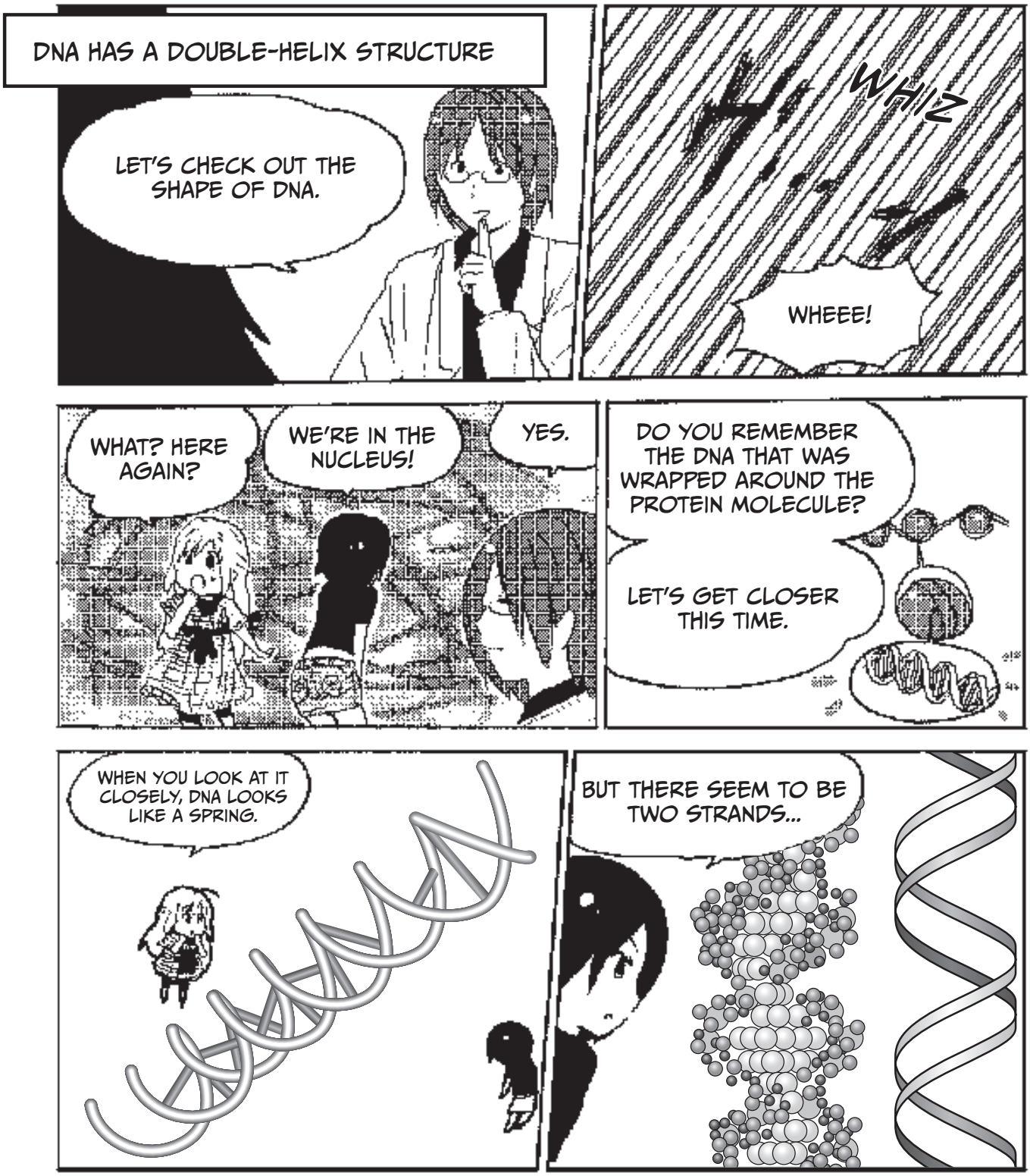

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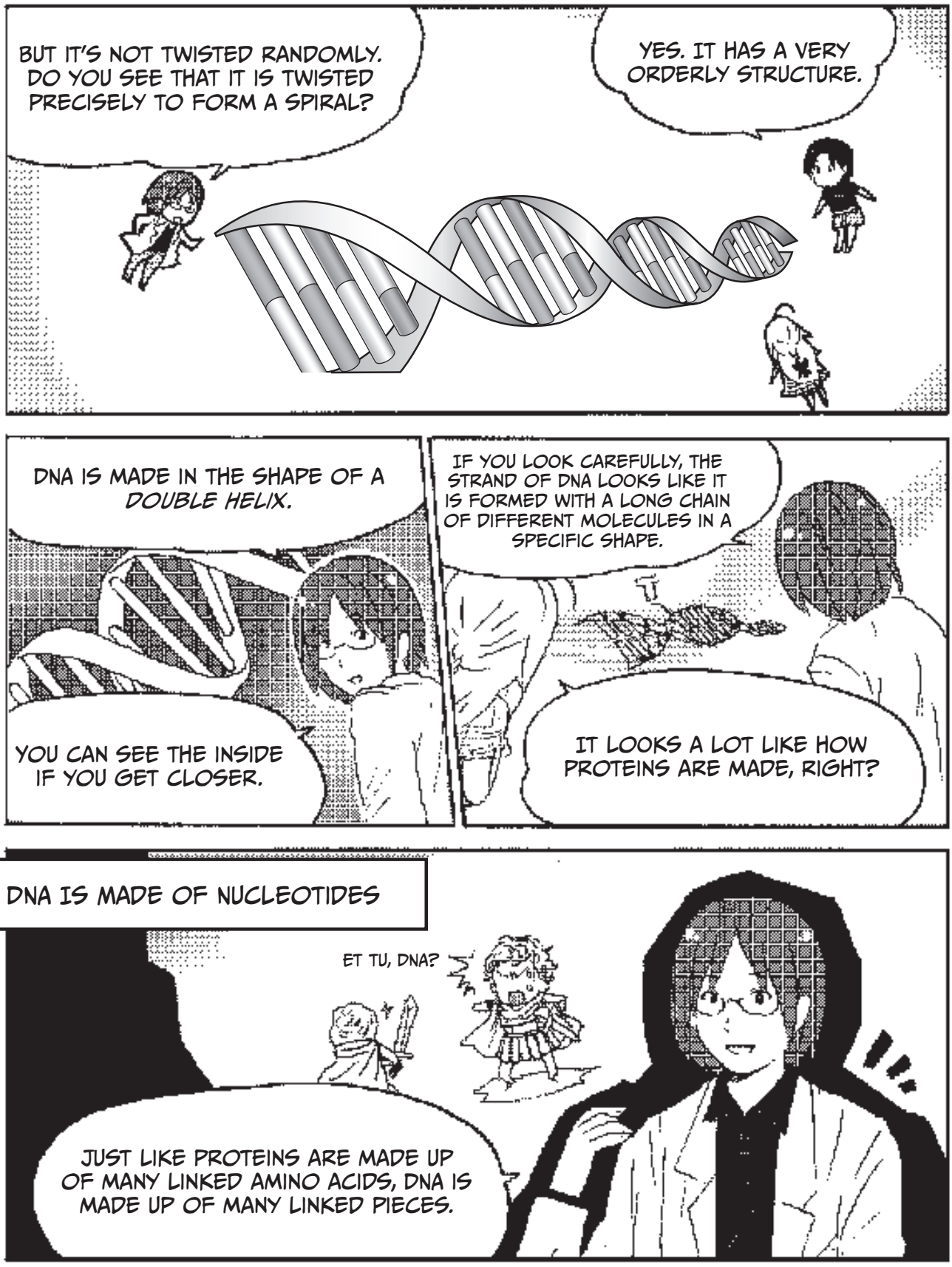

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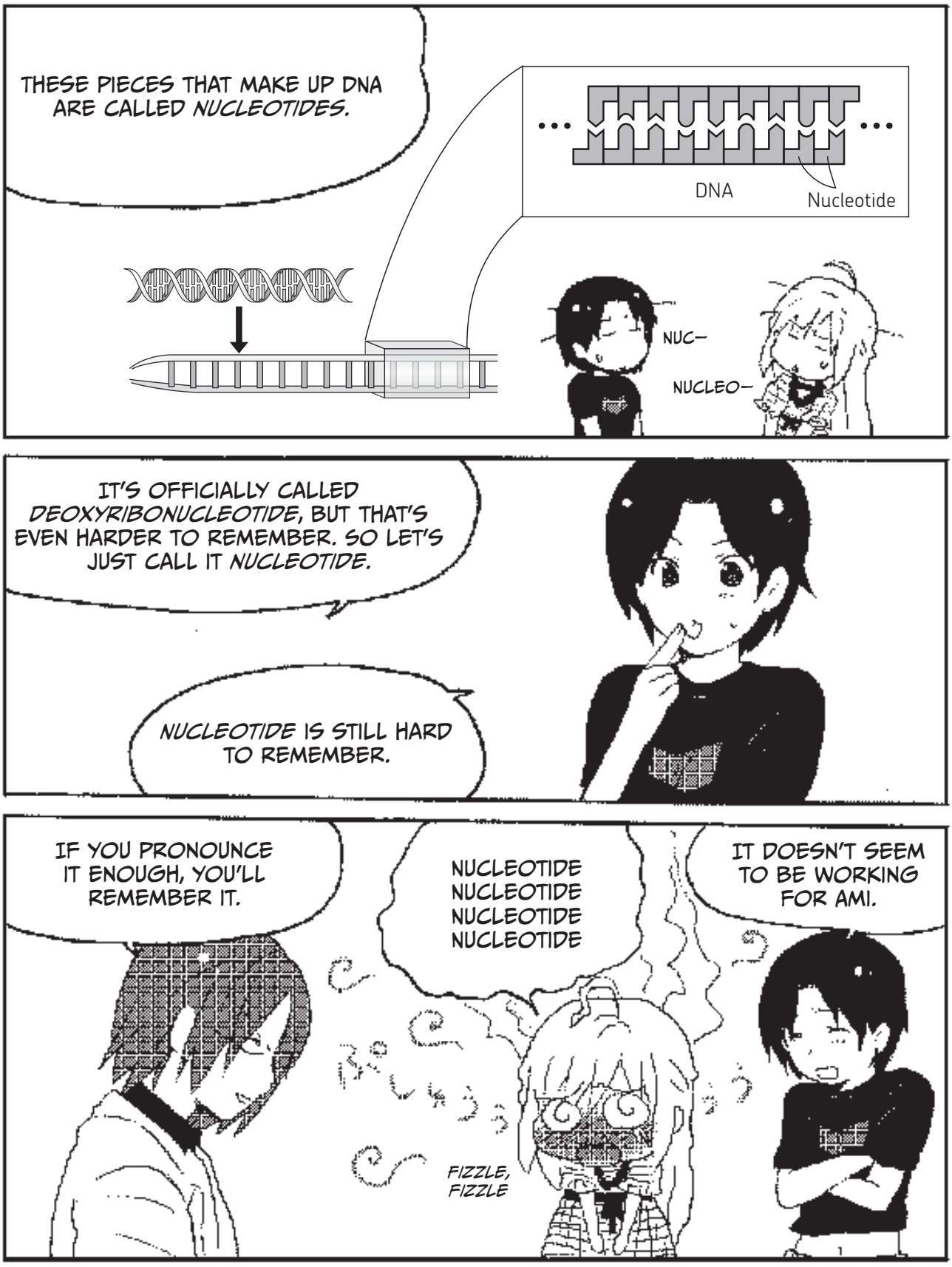

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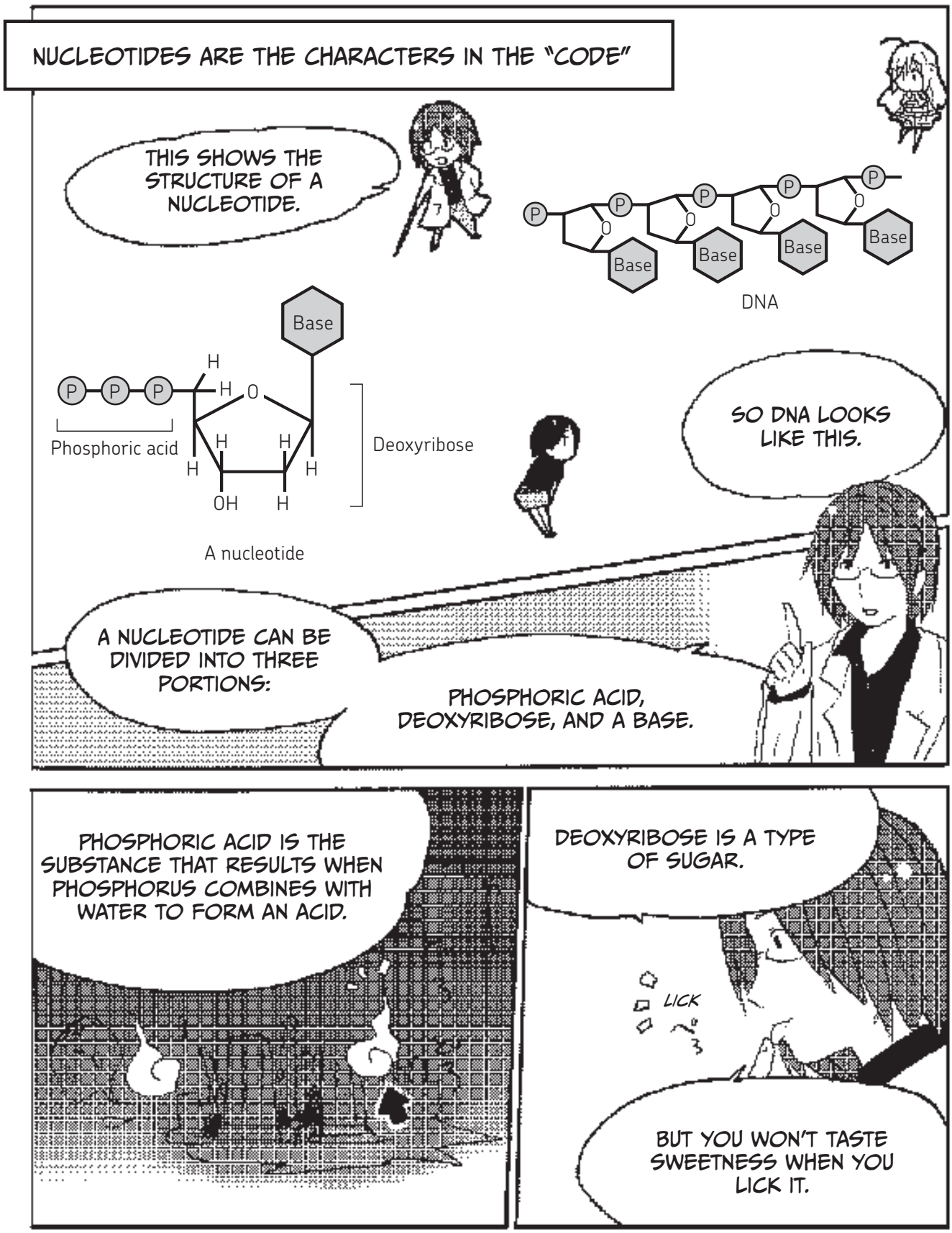

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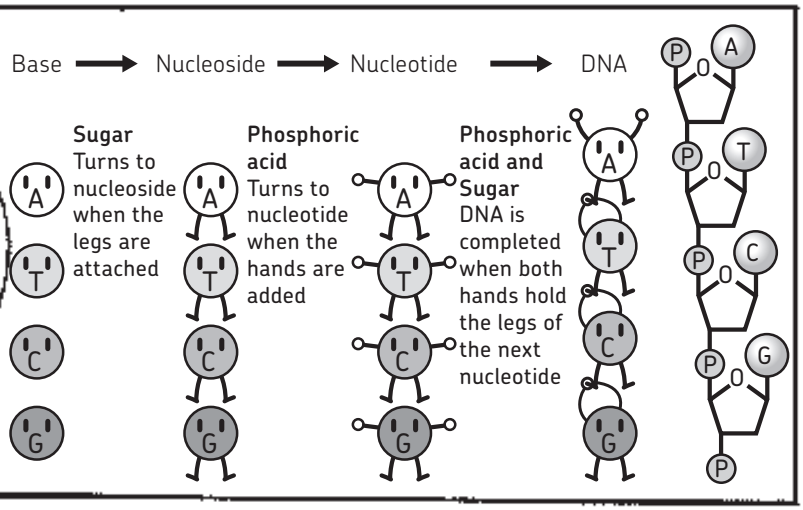
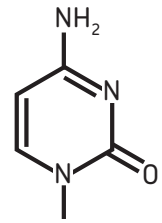
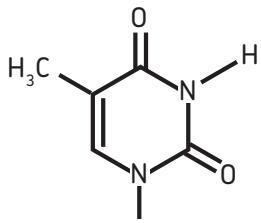
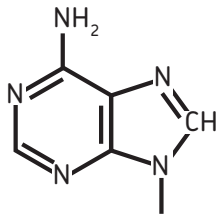
YOU CAN THINK OF THE BASE AS THE FACE OF A NUCLEOTIDE, DEOXYRIBOSE (SUGAR) AS THE LEGS, AND PHOSPHORIC ACID AS THE HANDS. NEIGHBORING NUCLEOTIDES ATTACH TO EACH OTHER, WITH THE HANDS OF ONE NUCLEOTIDE HOLDING THE LEGS OF ANOTHER, TO COMPLETE THE STRUCTURE OF DNA.

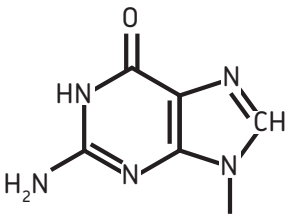


WHAT WE REALLY WANT TO FOCUS ON RIGHT NOW IS THE BASE. A NUCLEOTIDE CAN HAVE ONE OF FOUR TYPES OF BASES.


Cytosine (C)


Thymine (T)


Adenine (A)


Guanine (G)

ADENINE, GUANINE, CYTOSINE, AND THYMINE ARE OFTEN WRITTEN AS A, G, C, AND T, RESPECTIVELY.

THE BASE IS THE MOST IMPORTANT PART OF THE NUCLEOTIDE.

DNA IS MADE UP OF THESE FOUR TYPES OF NUCLEOTIDES CONNECTED IN A LONG LINE IN DIFFERENT ARRANGEMENTS.

THAT SOUNDS KIND OF FAMILIAR.

RIGHT! IT'S A LITTLE LIKE PROTEINS.

YES, PROTEINS ARE ALSO MADE BY STRINGING TOGETHER MOLECULES (THE 20 AMINO ACIDS).

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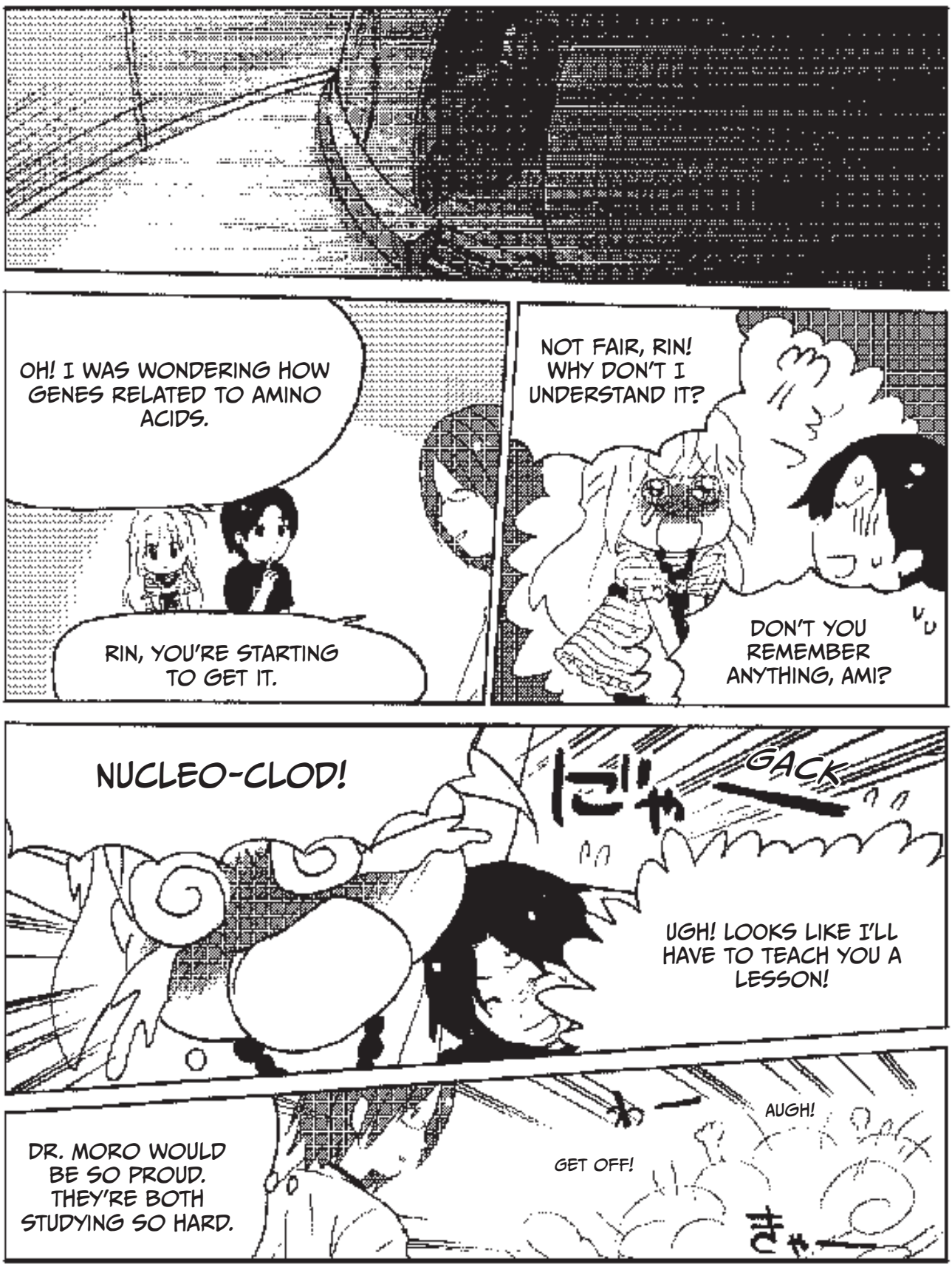

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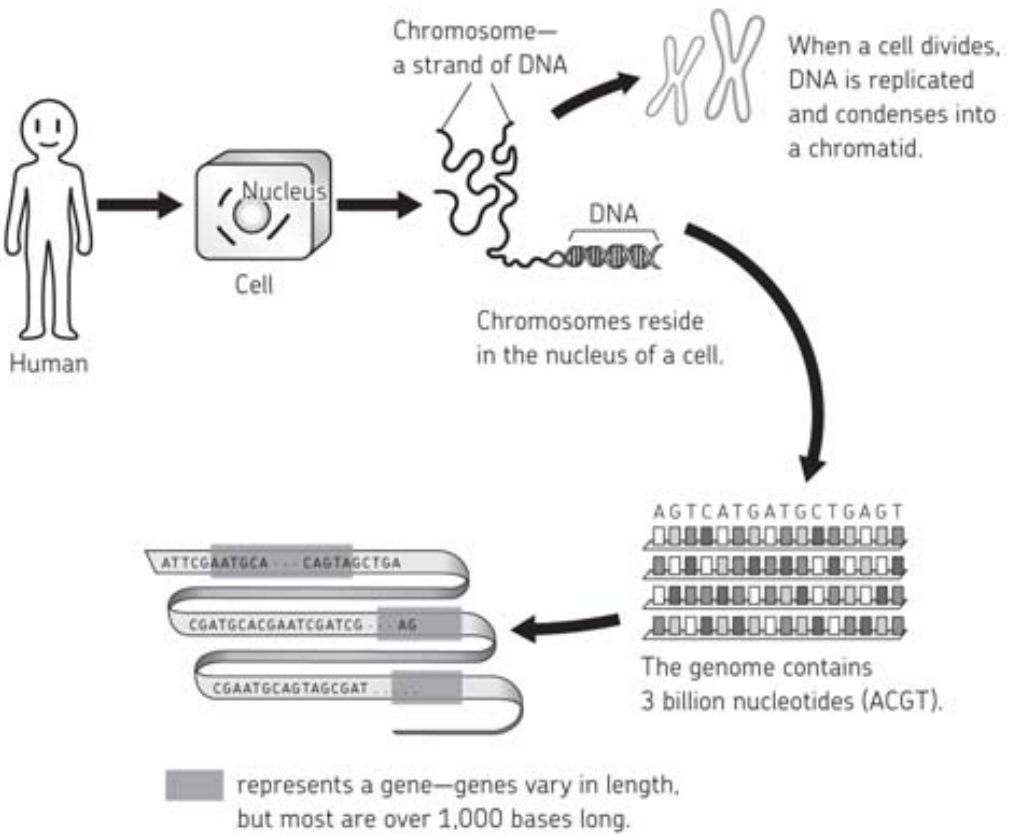
THE GENOME: A LIBRARY OF GENES

Knowing the sequence of every gene can be helpful in the fields of medicine and biology. In the future, people may be able to screen their DNA to see if they are predisposed for certain cancers or illnesses. A collection of the sequences of every strand of DNA in an organism is called a *genome*. Every living organism contains a genome in the nucleus of each cell.

The Human Genome Project was completed in 2003. This project found the sequence of bases in DNA and read every gene in the human body.

In human beings, this means looking at 30,000 genes, each with a unique long combination of the four bases A, G, C, and T.



Your cells have 46 pairs of chromosomes. One inherited from your mother, and the other from your father. Every cell has two sets of genes—the one exception is your sex cells, which only have one set of 23 chromosomes, a mixture from your mother and father. Sex cells are only used in reproduction.

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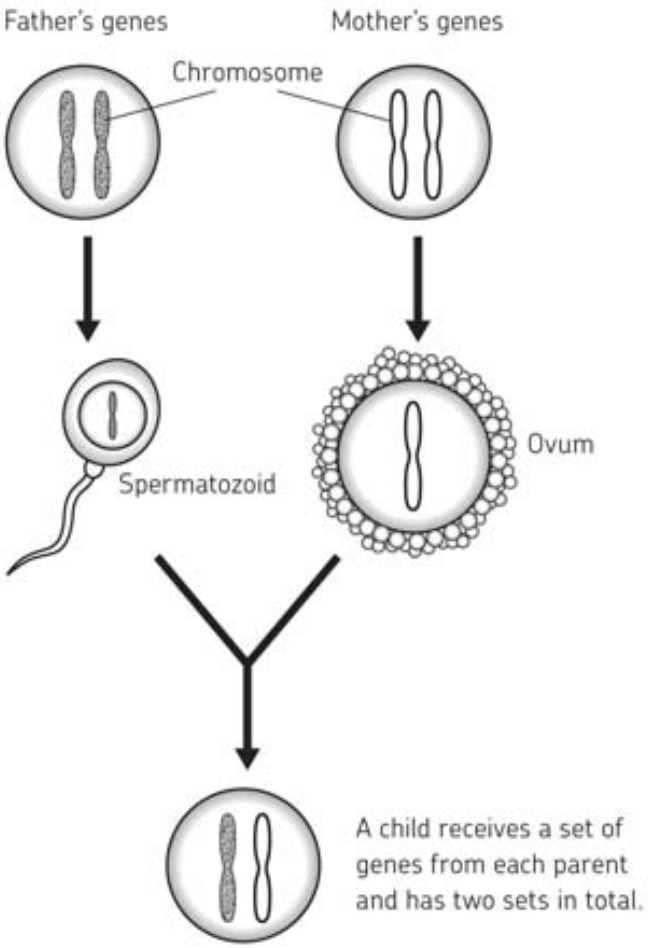

We actually have 23 chromosomes derived from our father and 23 from our mother (a total of 46 chromosomes made up of 23 pairs). A single pair of chromosomes is shown above.

A genome can be compared to a library containing books of short stories. Each book is a chromosome; each story is a gene about how to make one protein.

But genomes contain more than just genes. There are base pairs in between genes that do not code for genes. Research is currently going on to learn more about these parts of the genome. Noncoding sections of DNA can be important for functions such as regulating the expression of genes.

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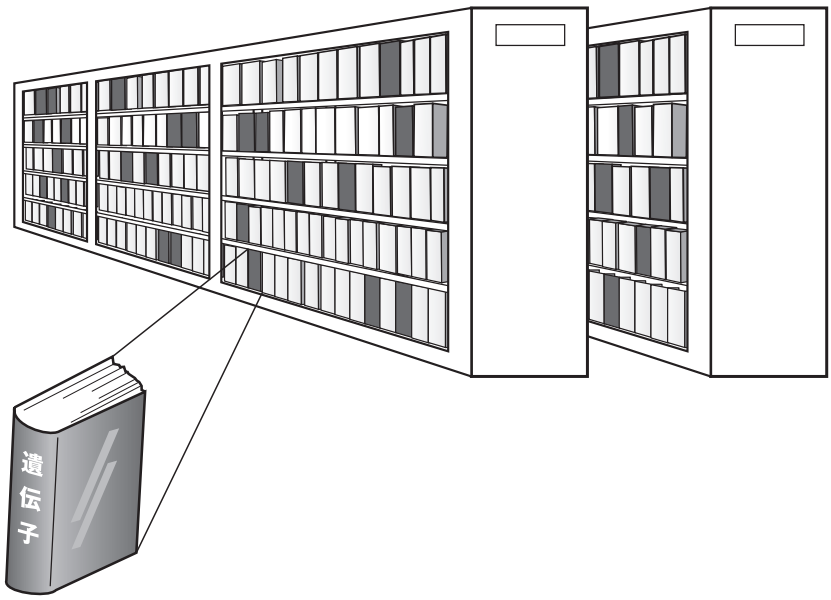
If every base is equivalent to a letter, the genome would be over 100 million words long. That's equivalent to a library of 5,000 books, each 300 pages long; the entire library fits into a cell nucleus the size of a pinpoint. A complete copy of the library (all 5,000 volumes) is contained in almost every cell.



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3

DNA REPLICATION AND CELL DIVISION

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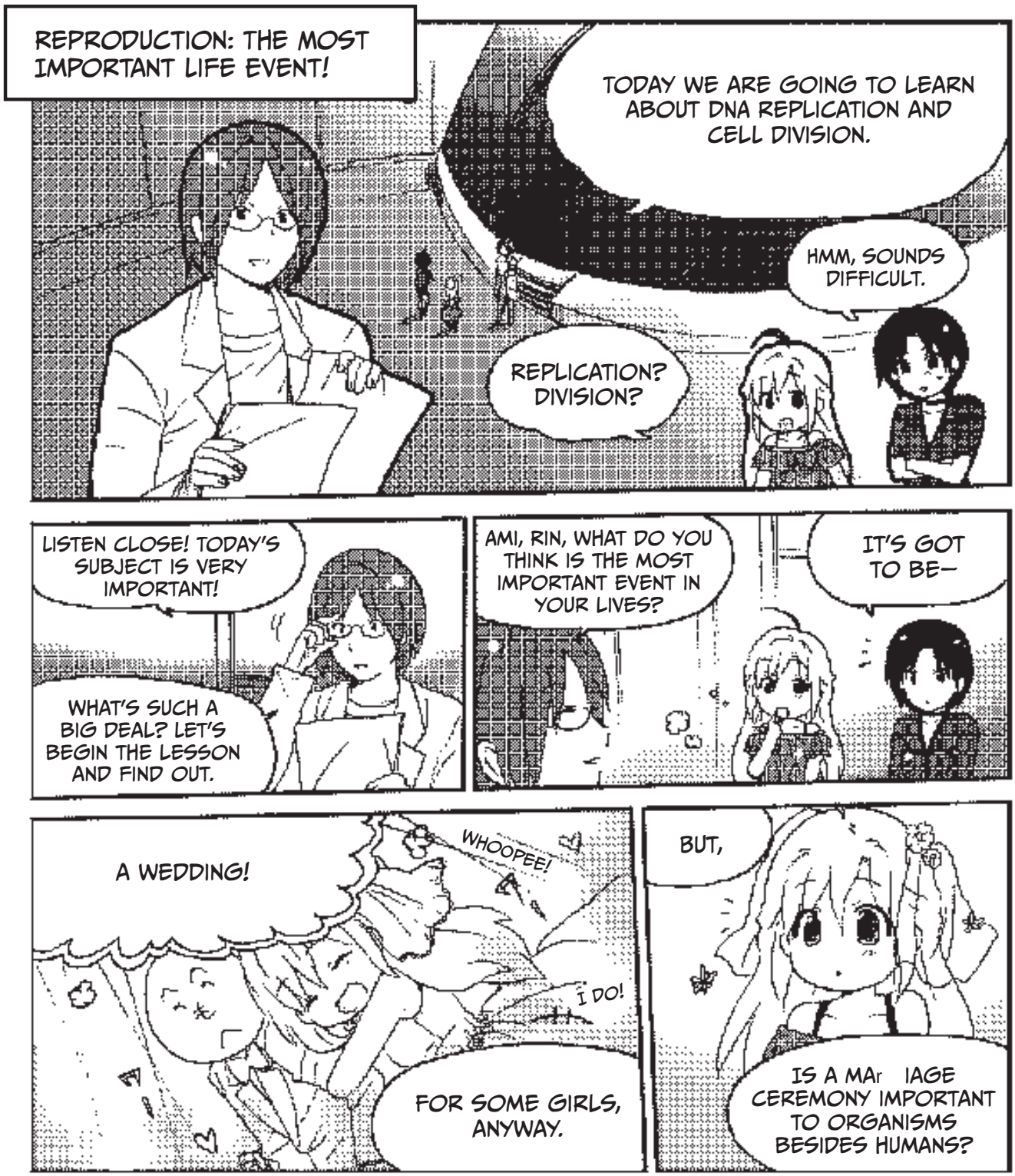
CELLS MULTIPLY THROUGH DIVISION



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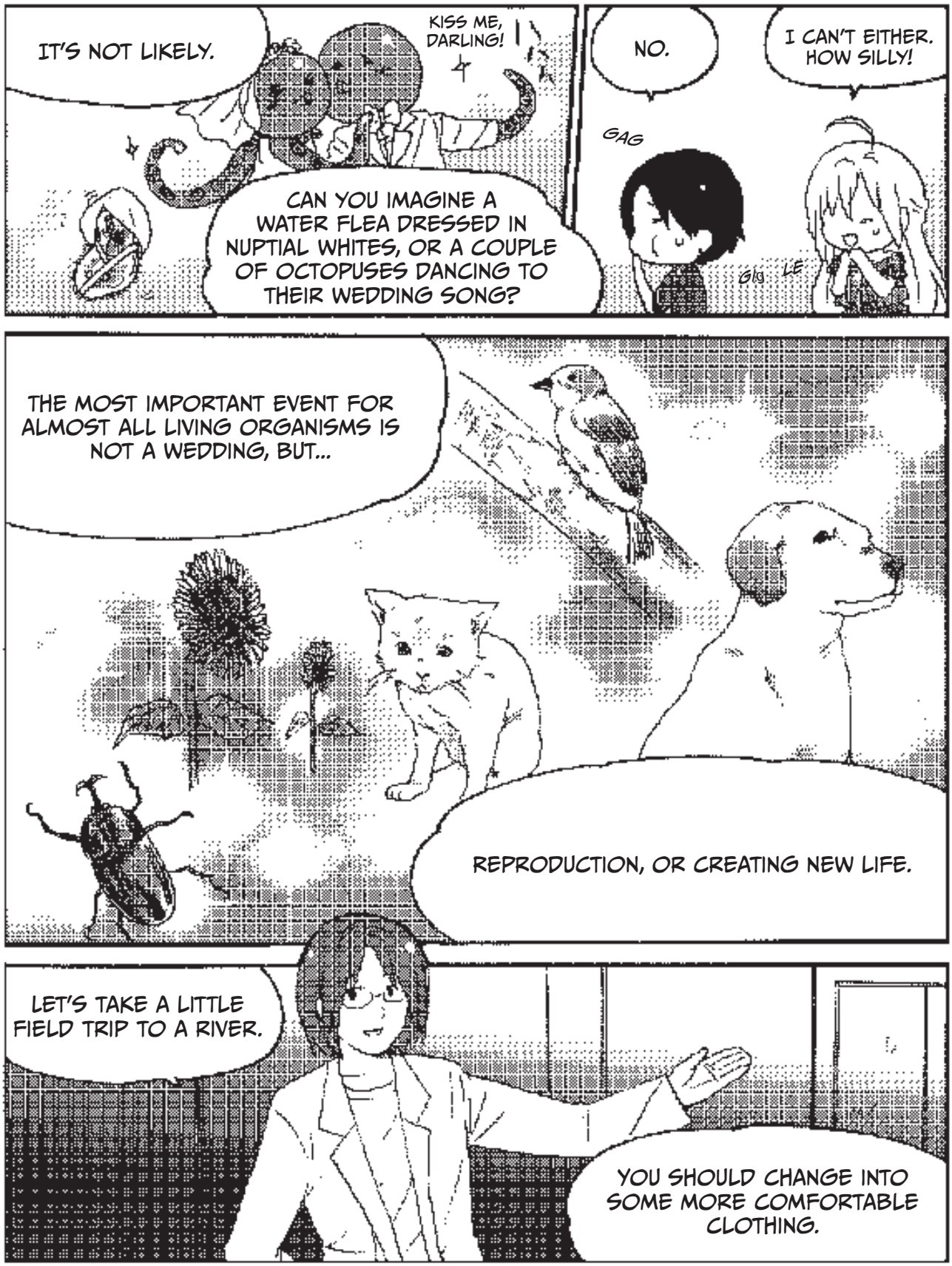

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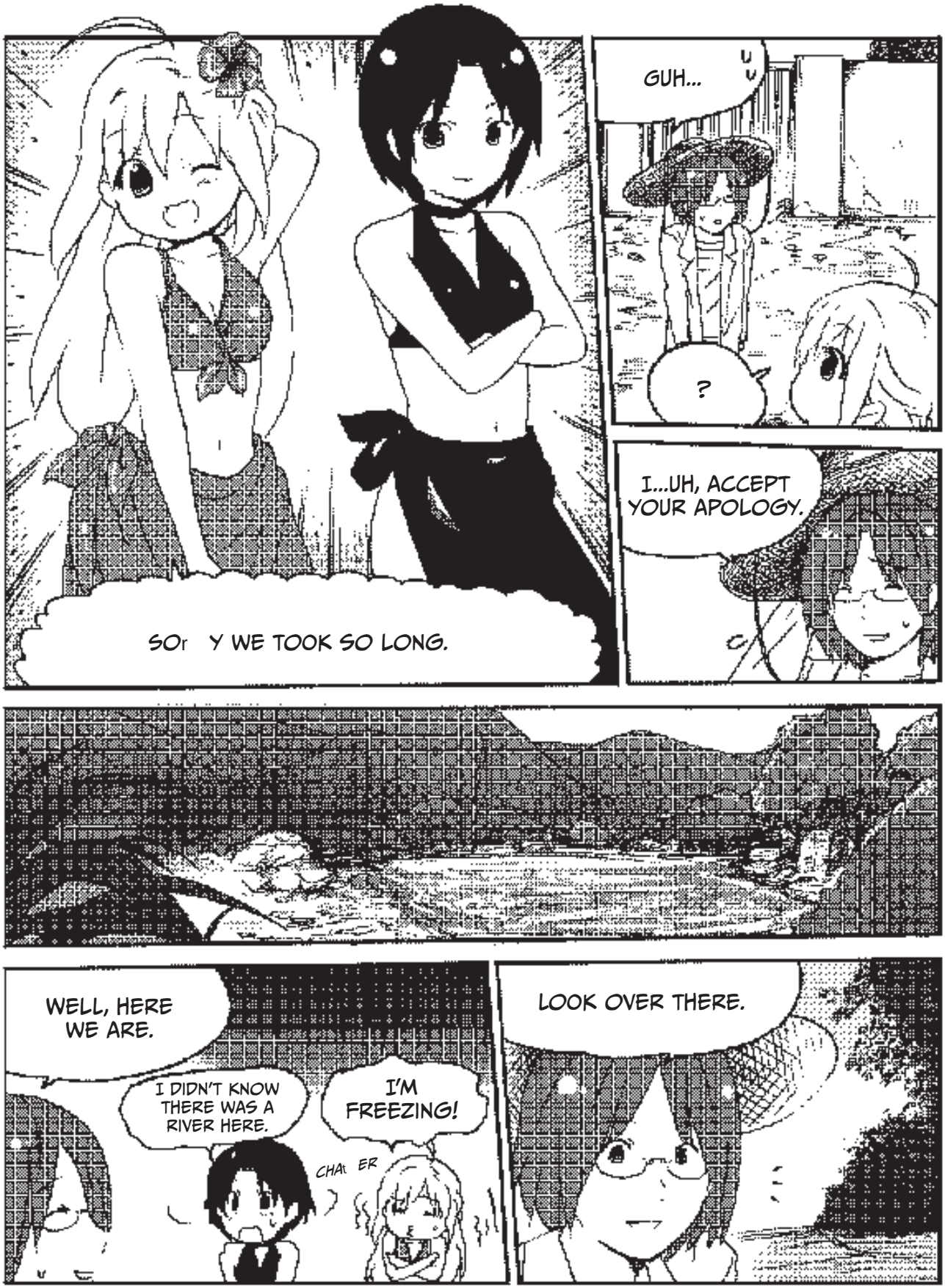

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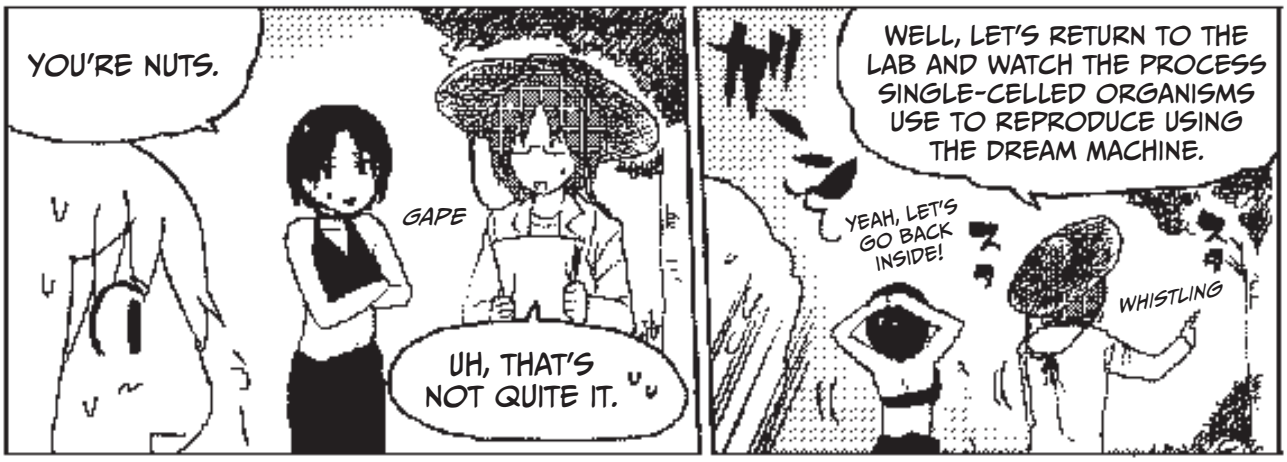
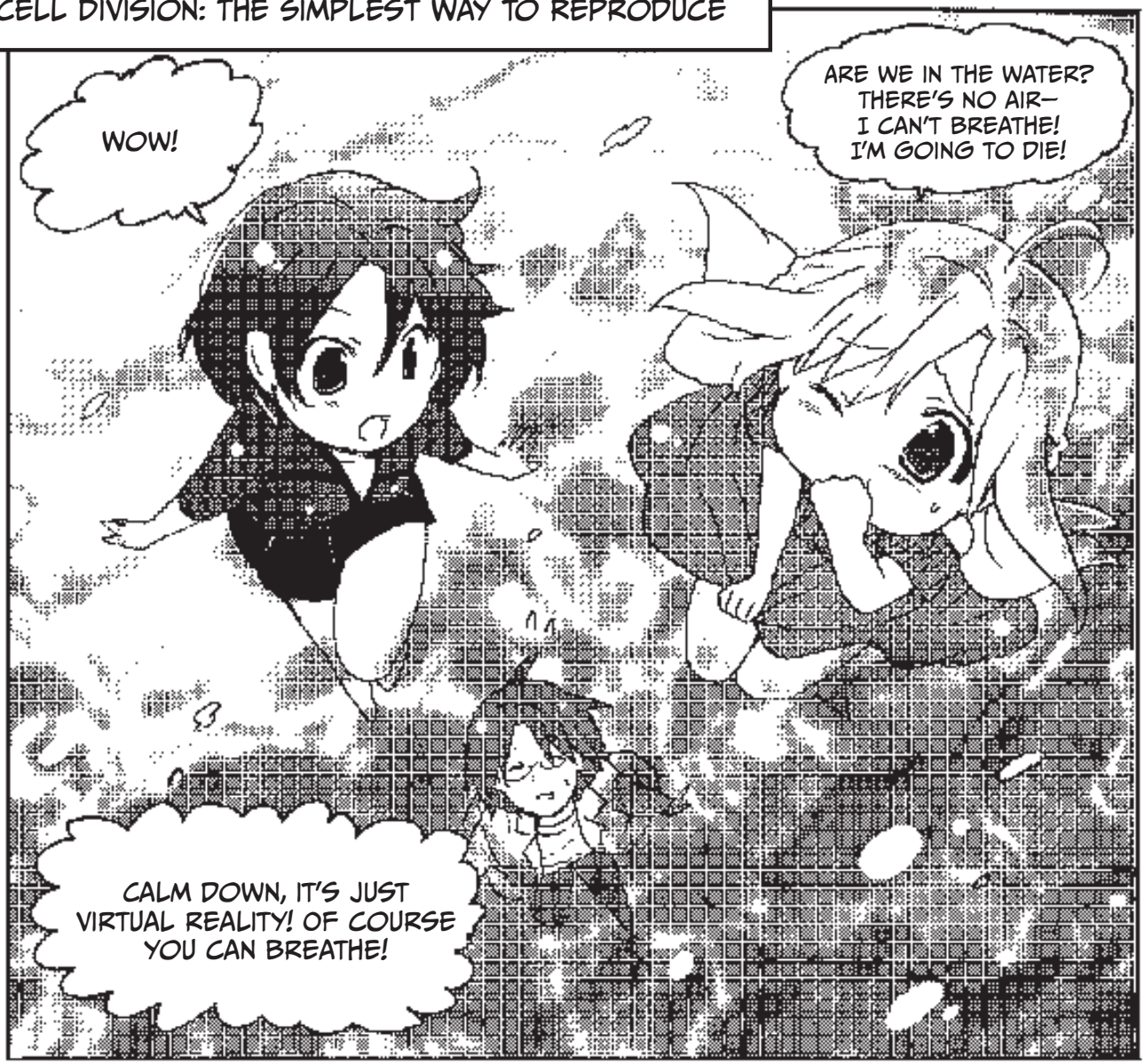


CELL DIVISION: THE SIMPLEST WAY TO REPRODUCE



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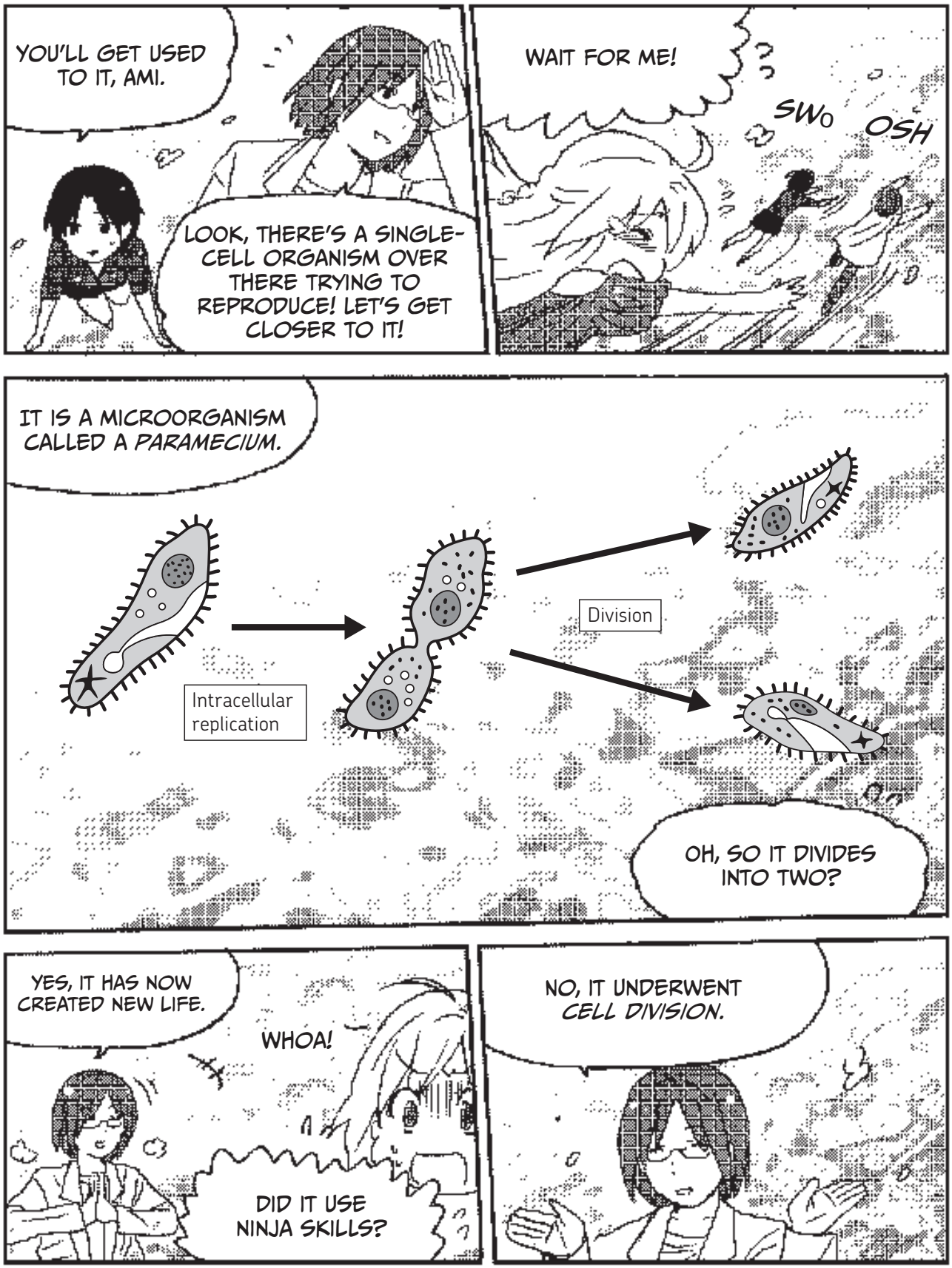

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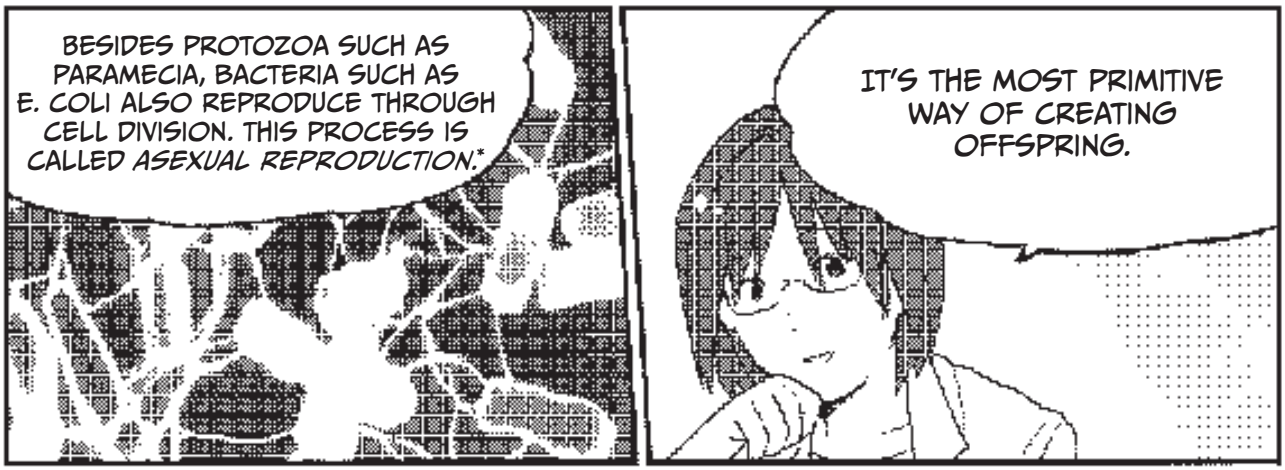
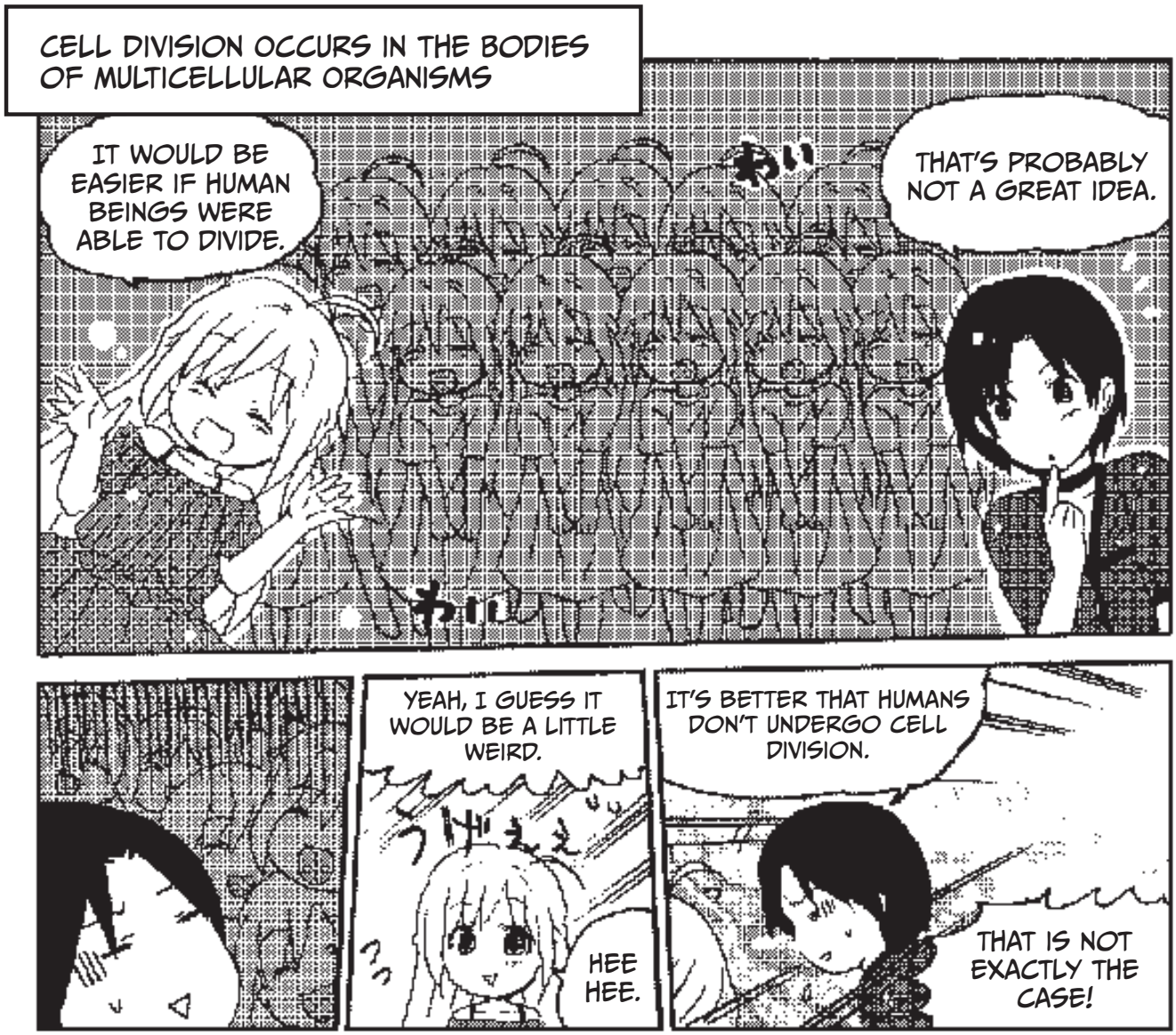


* THERE ARE SOME EXCEPTIONS TO THIS RULE. SOME SINGLE-CELLED ORGANISMS, INCLUDING PARAMECIA, EXCHANGE SOME OF THEIR GENES WITHOUT REPRODUCING.



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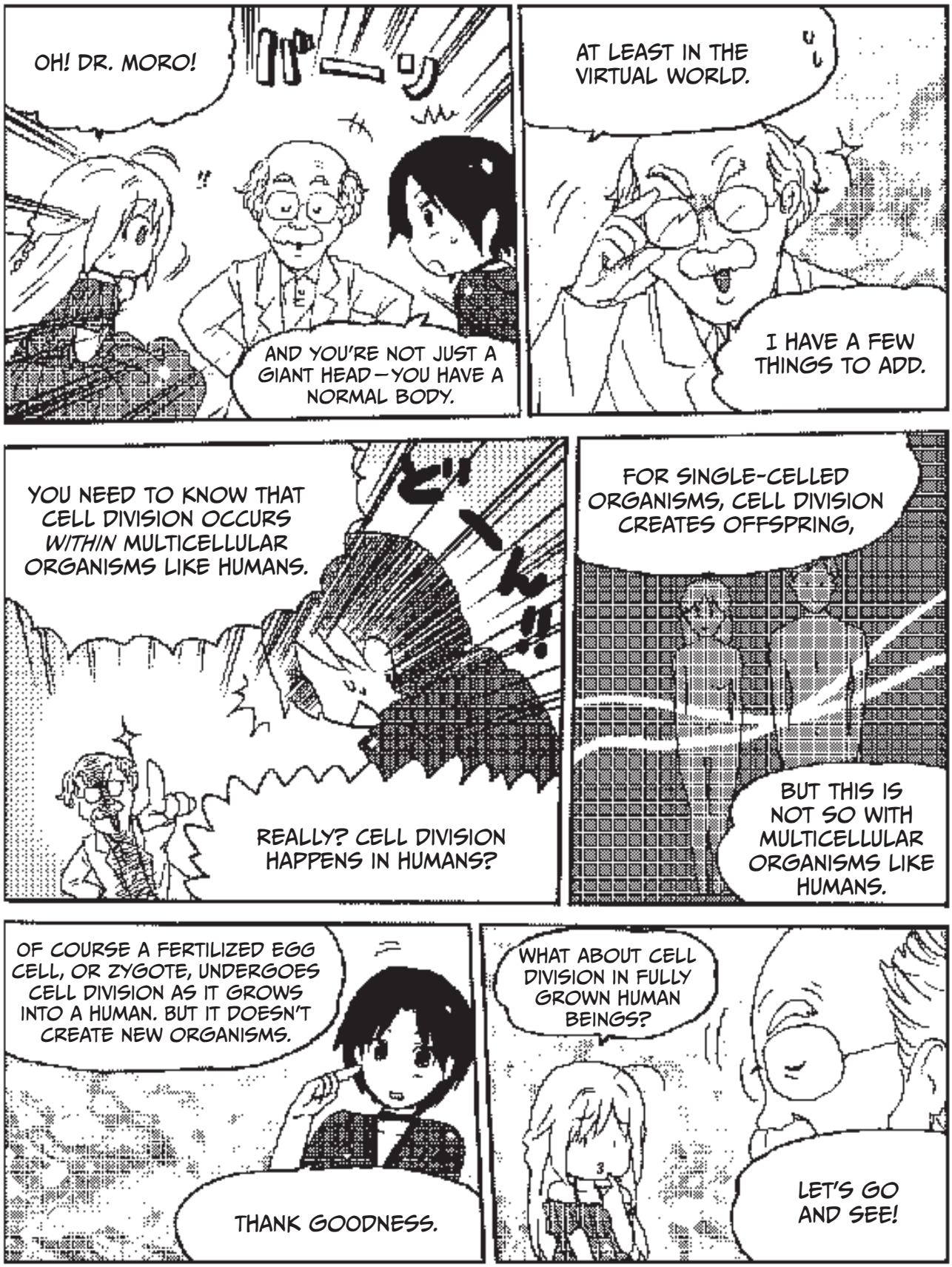

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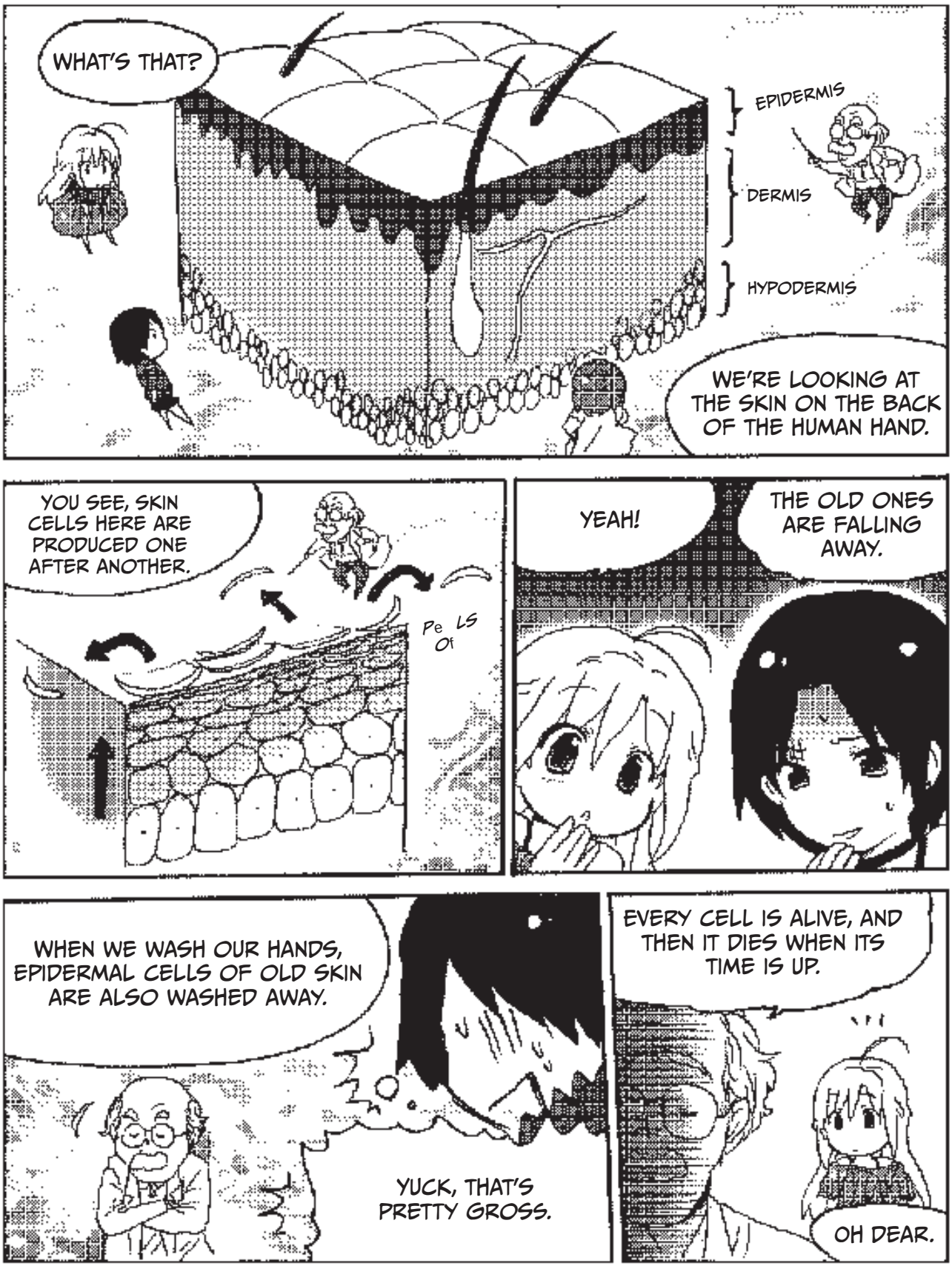

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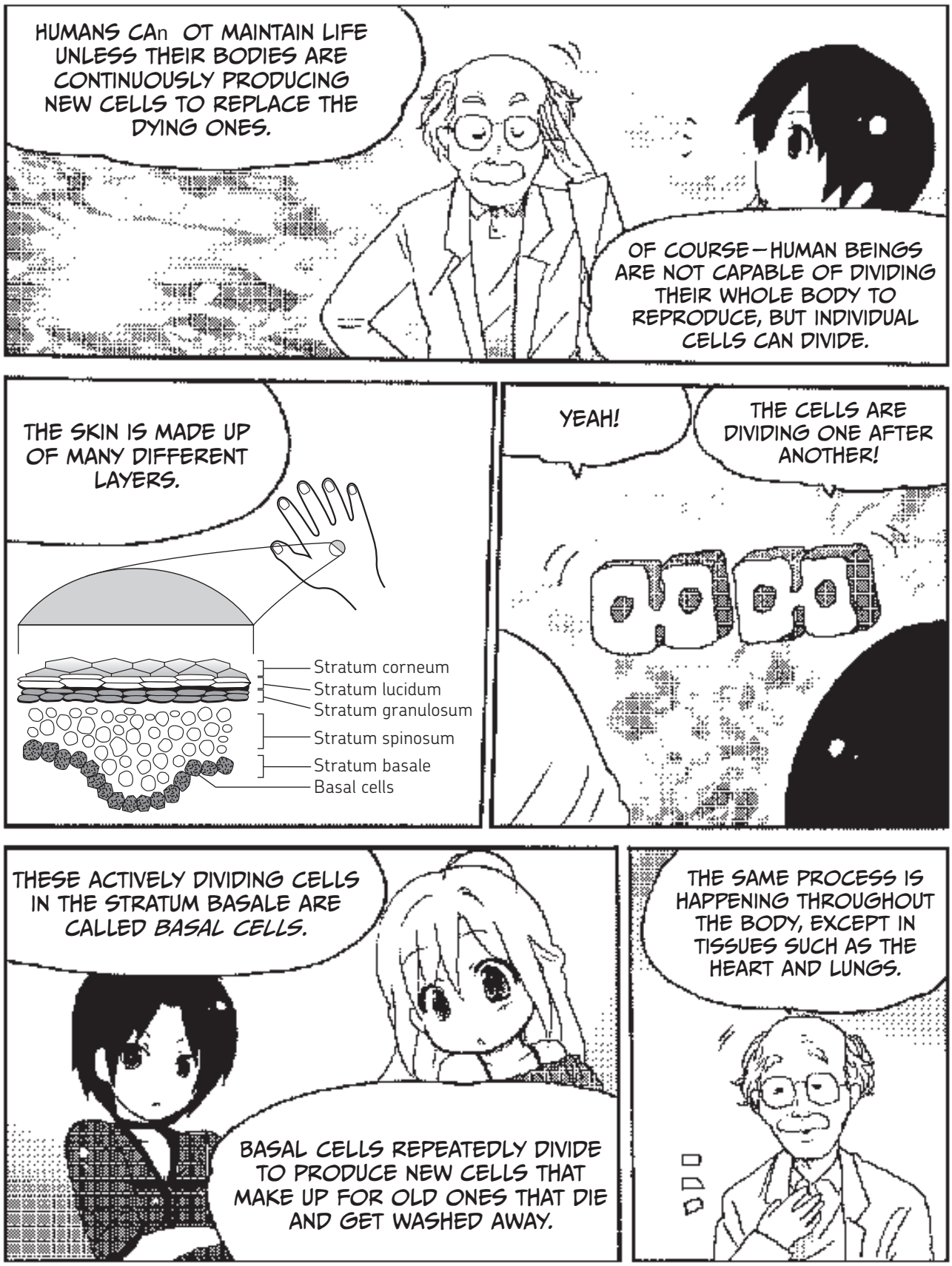

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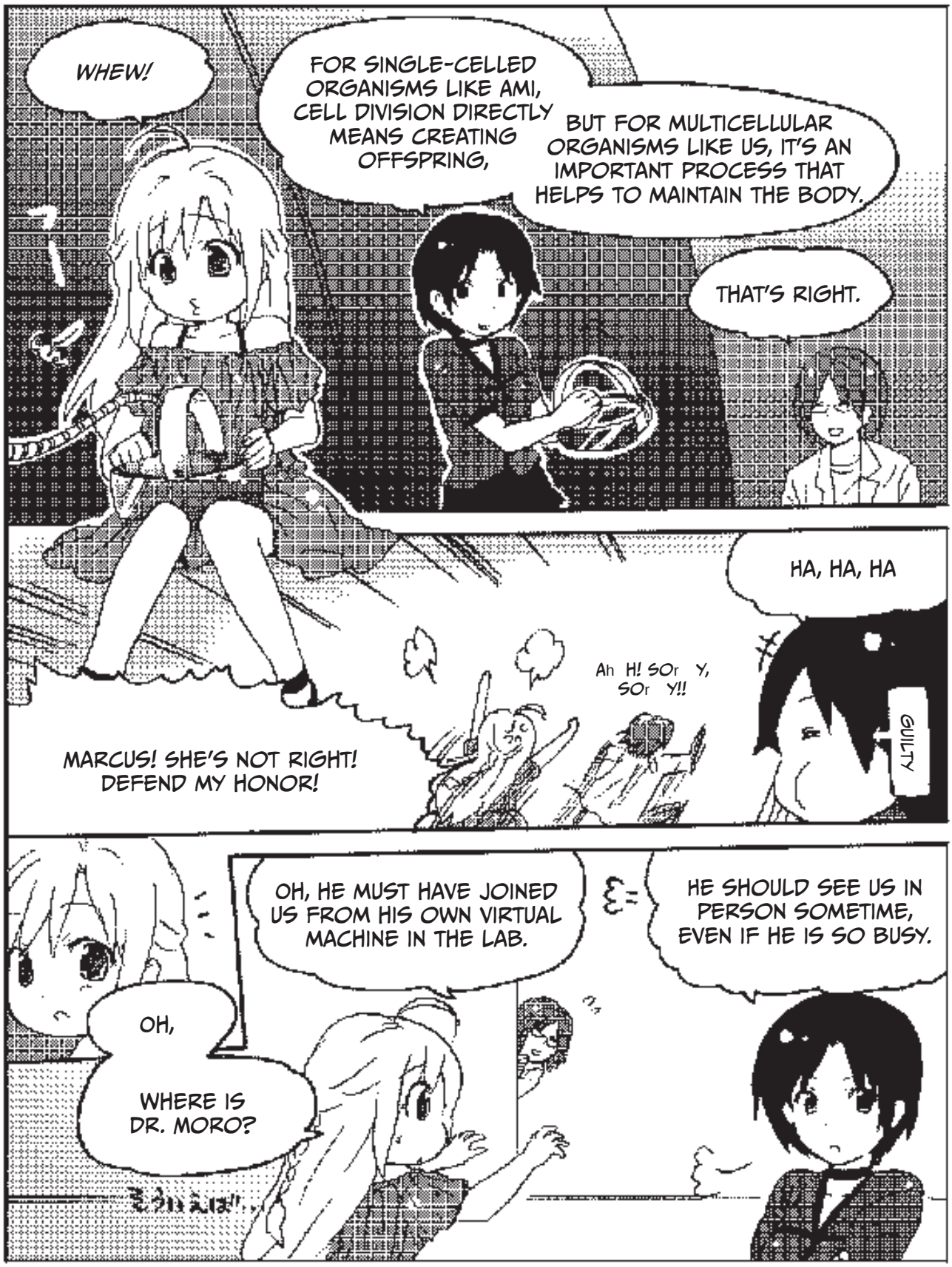

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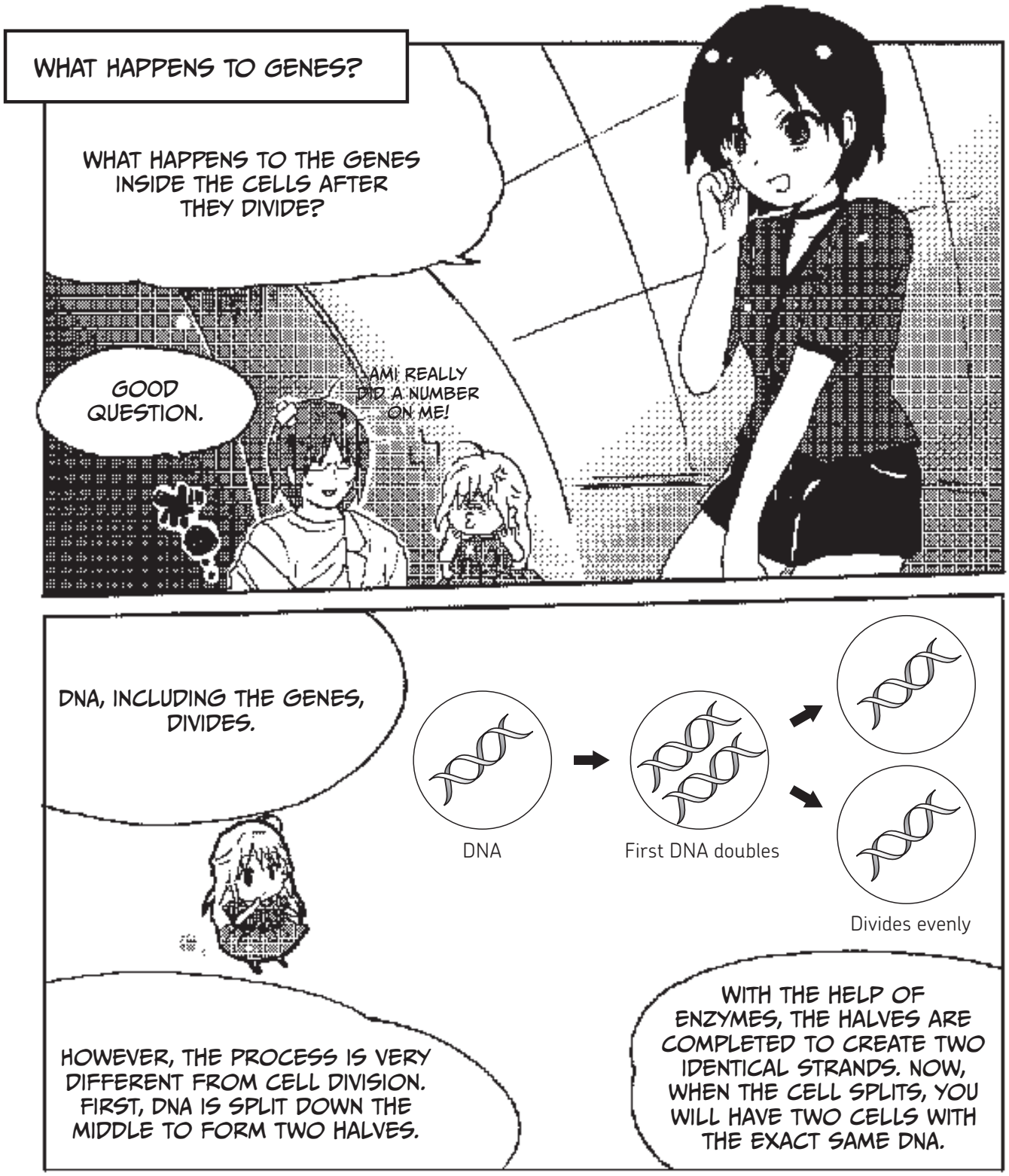
DNA IS REPLICATED BEFORE CELL DIVISION



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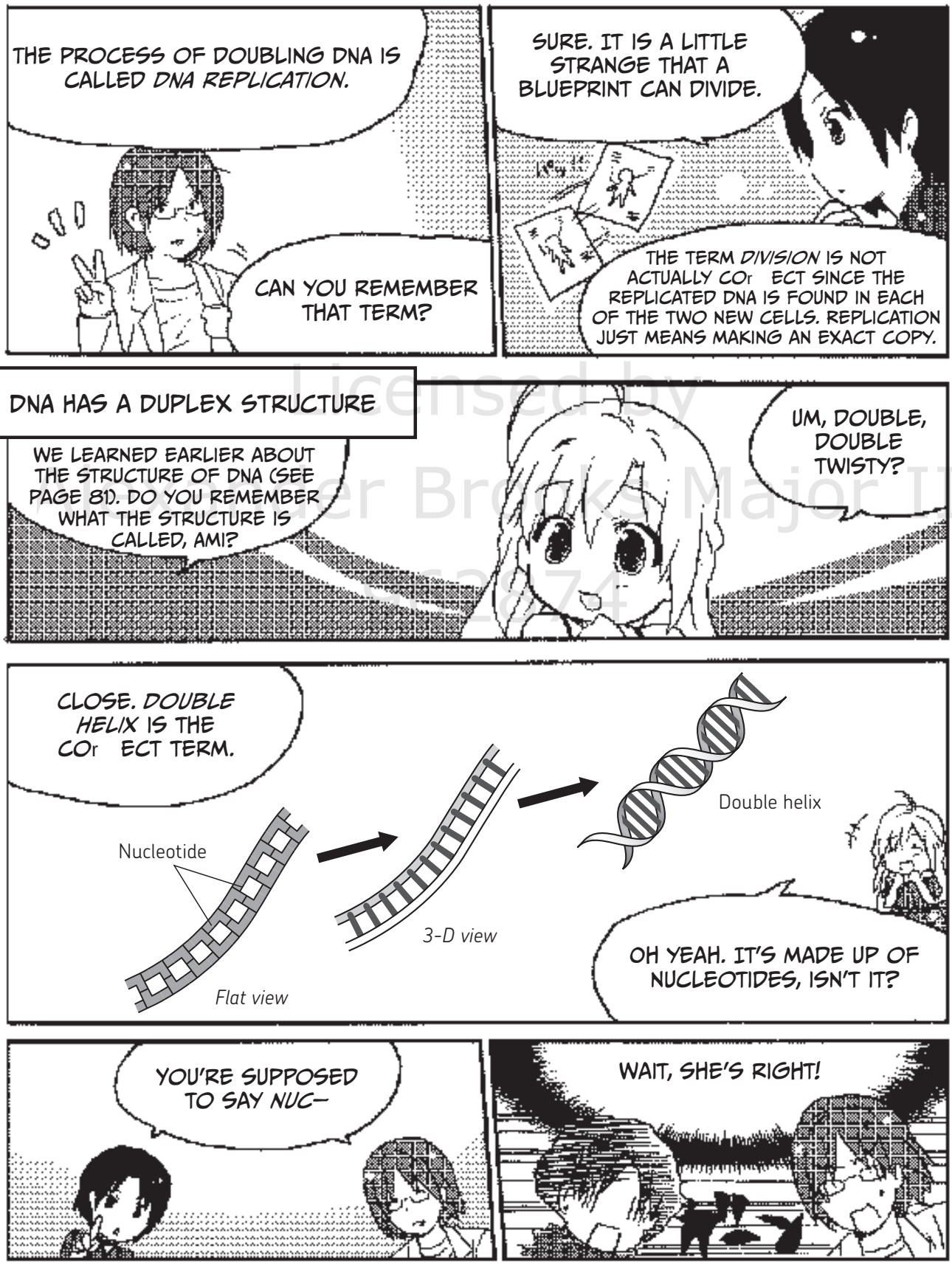

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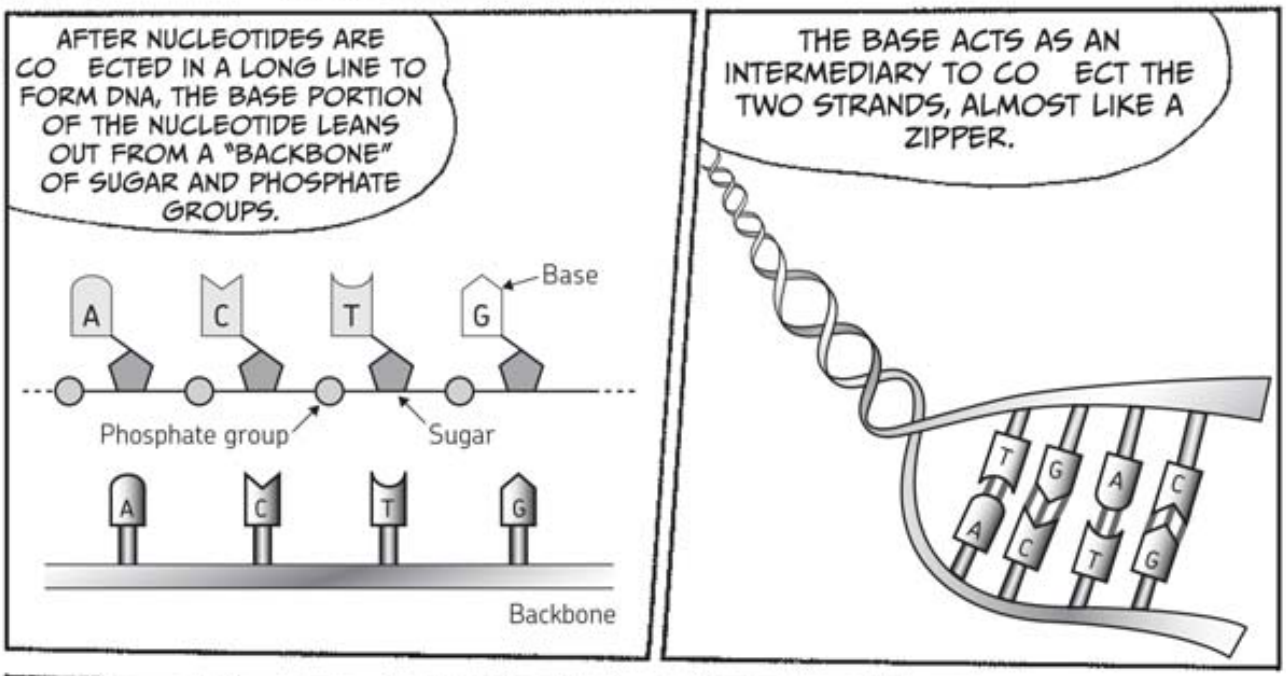

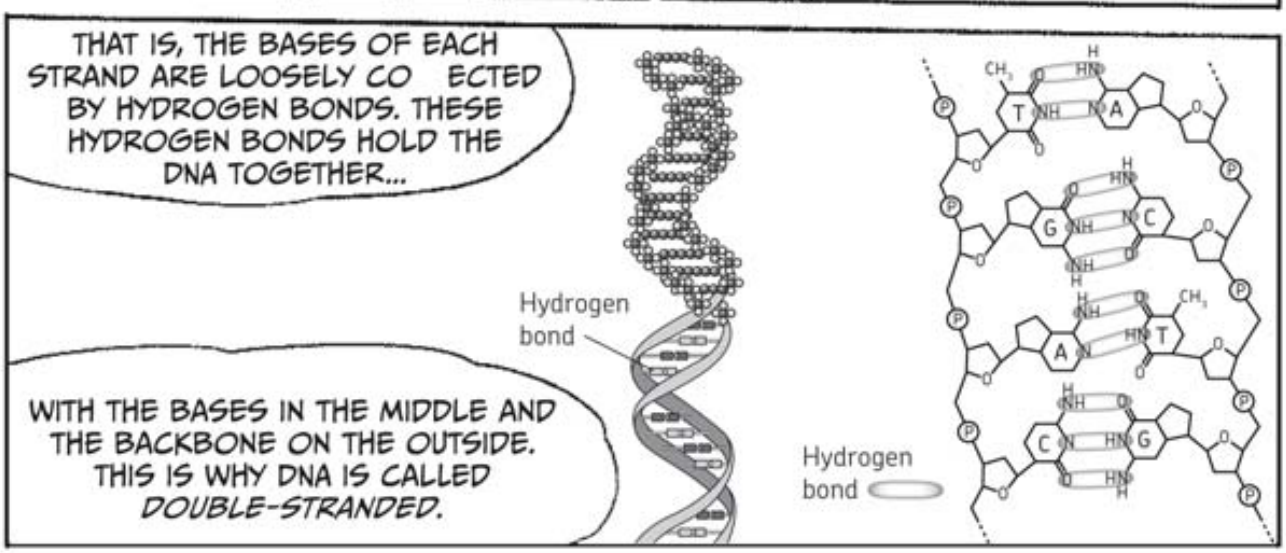

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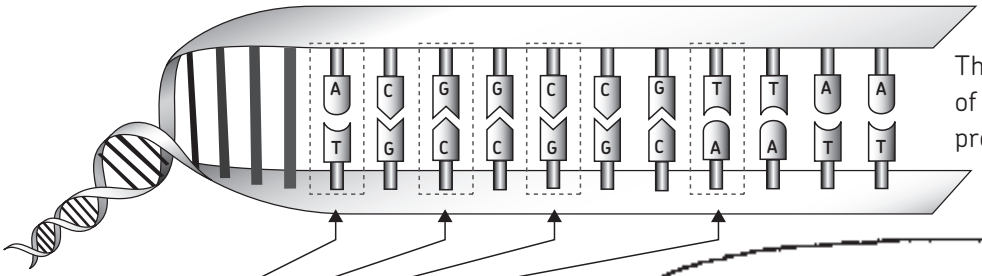
IT'S IMPORTANT TO KNOW THAT A BASE CAN ONLY INTERACT WITH ONE OTHER BASE AND THAT ITS PARTNER IS ALWAYS THE SAME. ADENINE (A) ALWAYS BINDS THYMINE (T) AND GUANINE (G) ALWAYS BINDS TO CYTOSINE (C).



The partner of each base is predetermined.

If one base is A (adenine), its partner is always T (thymine). If one base is G (guanine), its partner is always C (cytosine).

THIS MEANS THAT IF THE DNA SEQUENCE OF A BASE IS ACGGCCGTTa , THE DNA SEQUENCE OF THE PARTNER IN THE DOUBLE HELIX IS ALWAYS TGCCGGCa TT.

SO IF WE KNOW THE DNA SEQUENCE OF ONE STRAND, WE KNOW THE SEQUENCE OF ITS PARTNER.

OH, YES!

TRY TO REMEMBER THIS, SINCE IT PLAYS AN IMPORTANT ROLE IN THE REPLICATION OF DNA.

DNA POLYMERASE'S ROLE IN DNA REPLICATION

INSIDE THE NUCLEUS

OH! DNA REPLICATION HAS STARTED!

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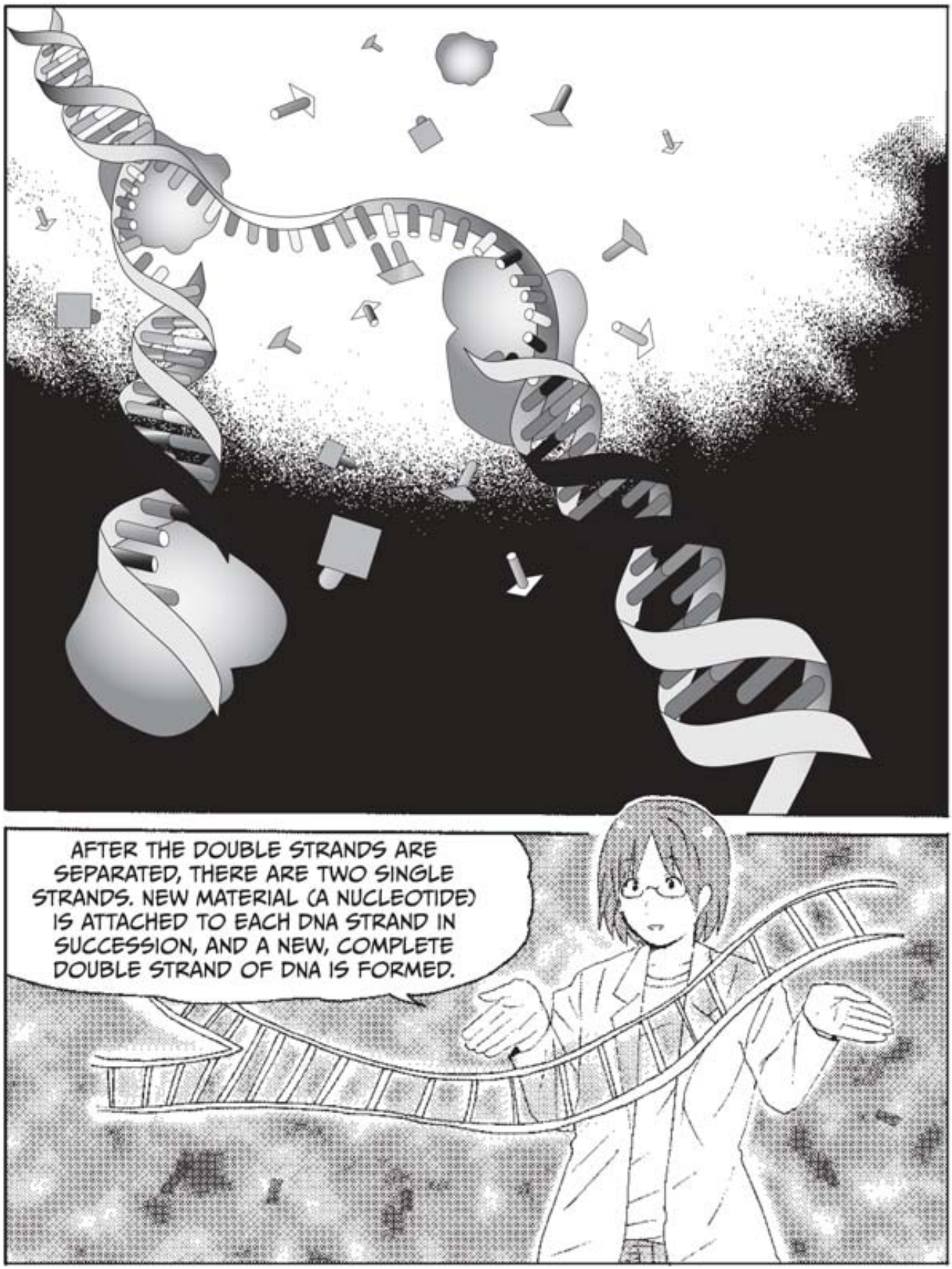

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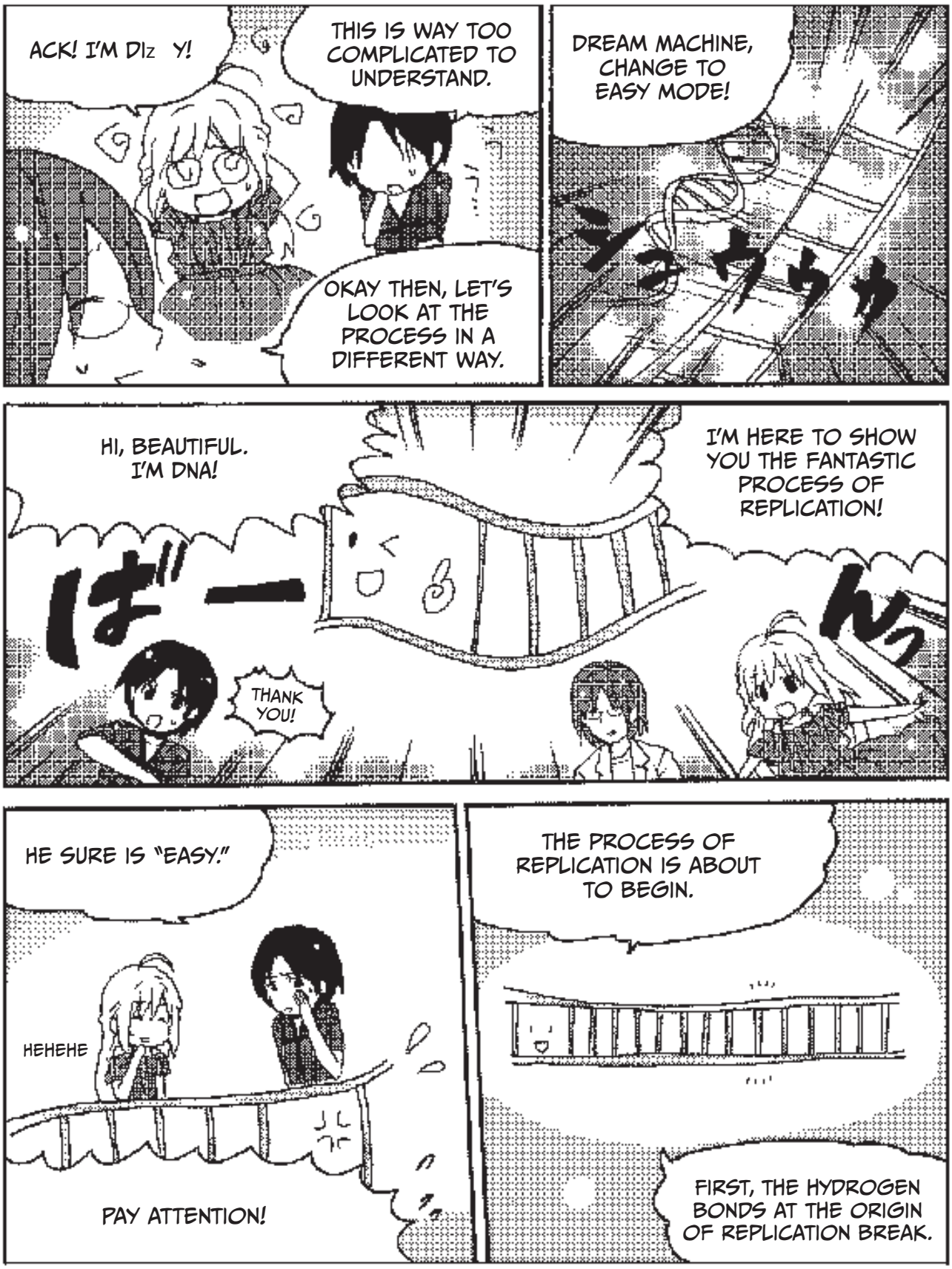

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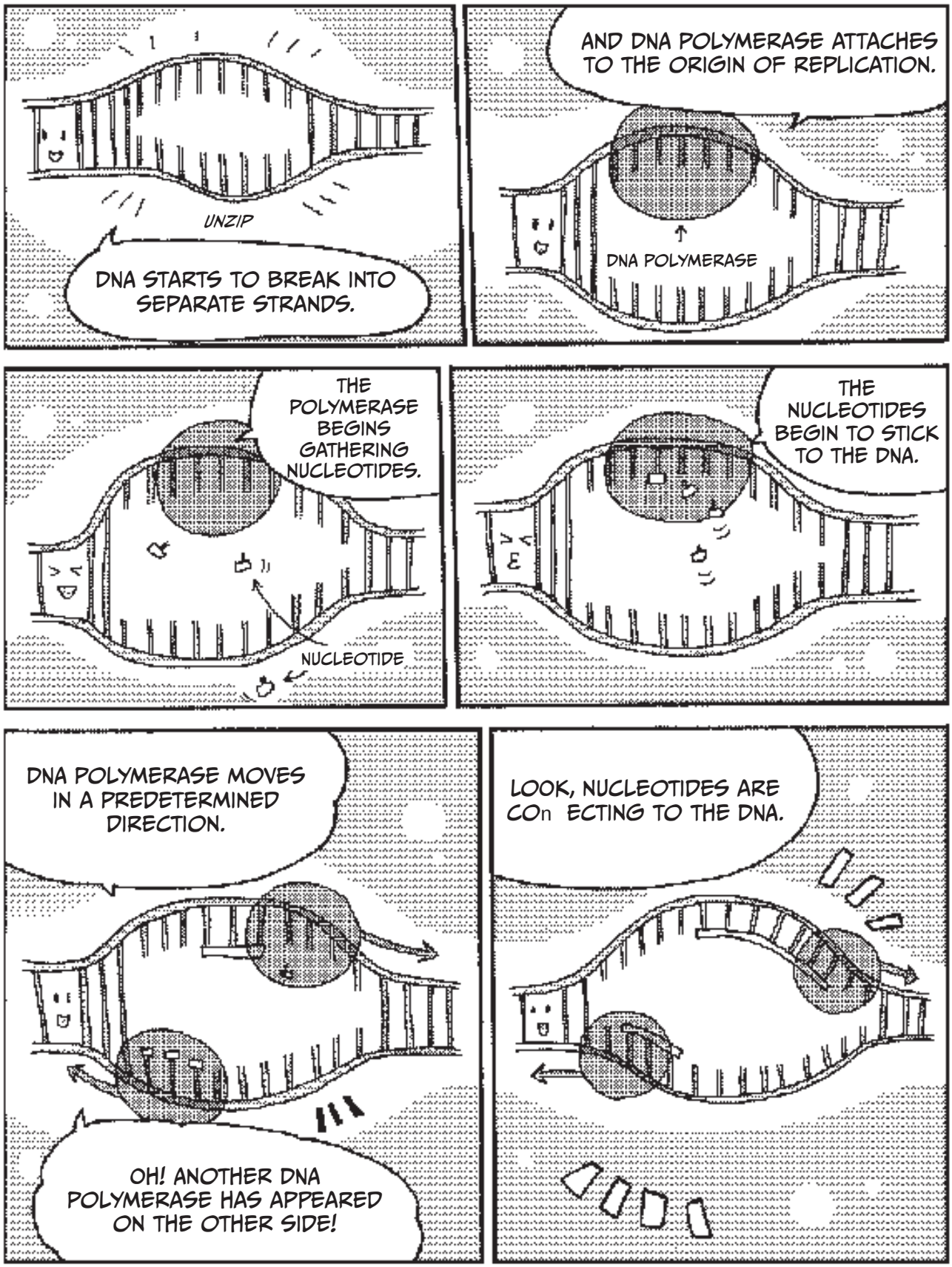

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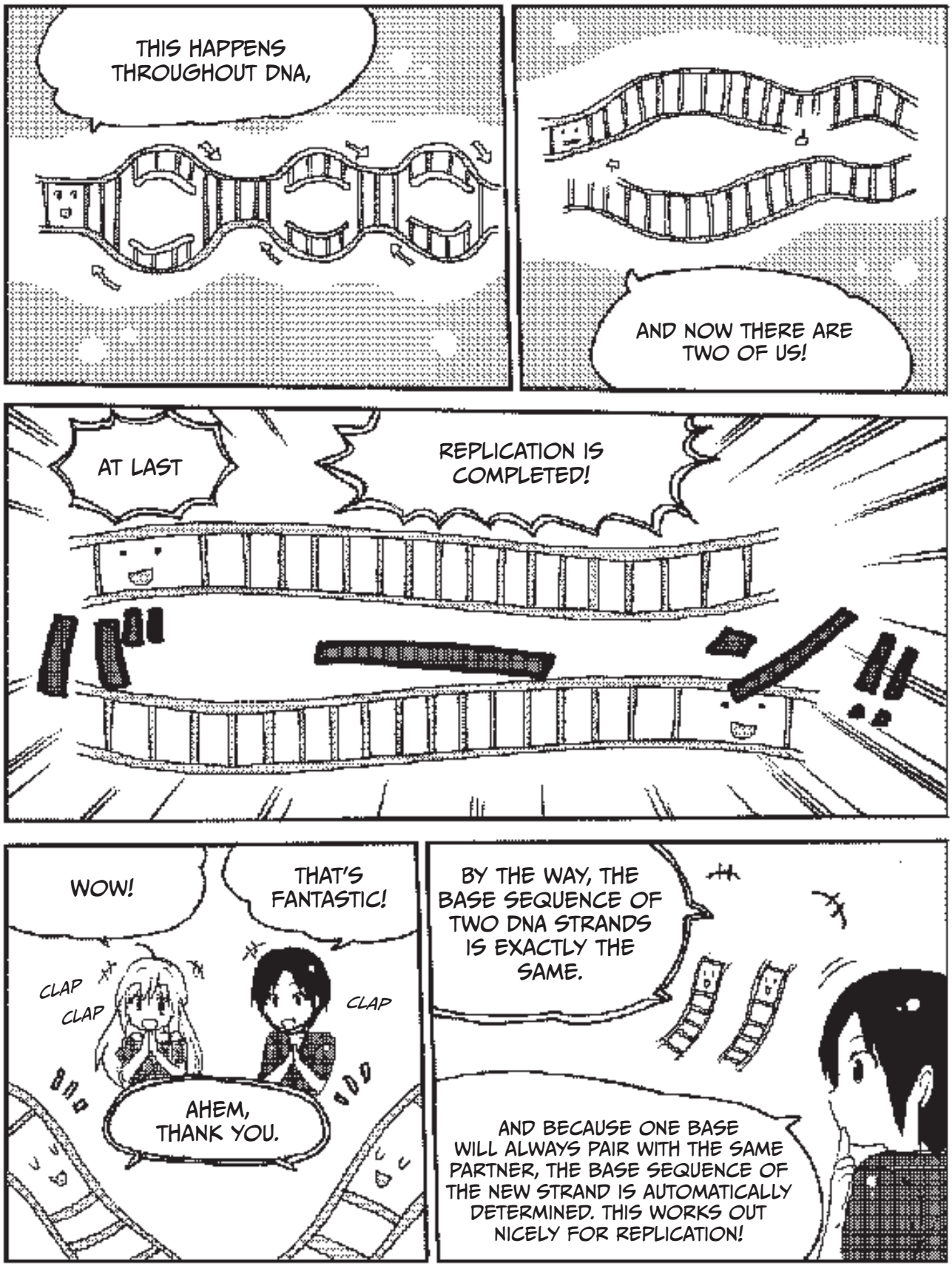

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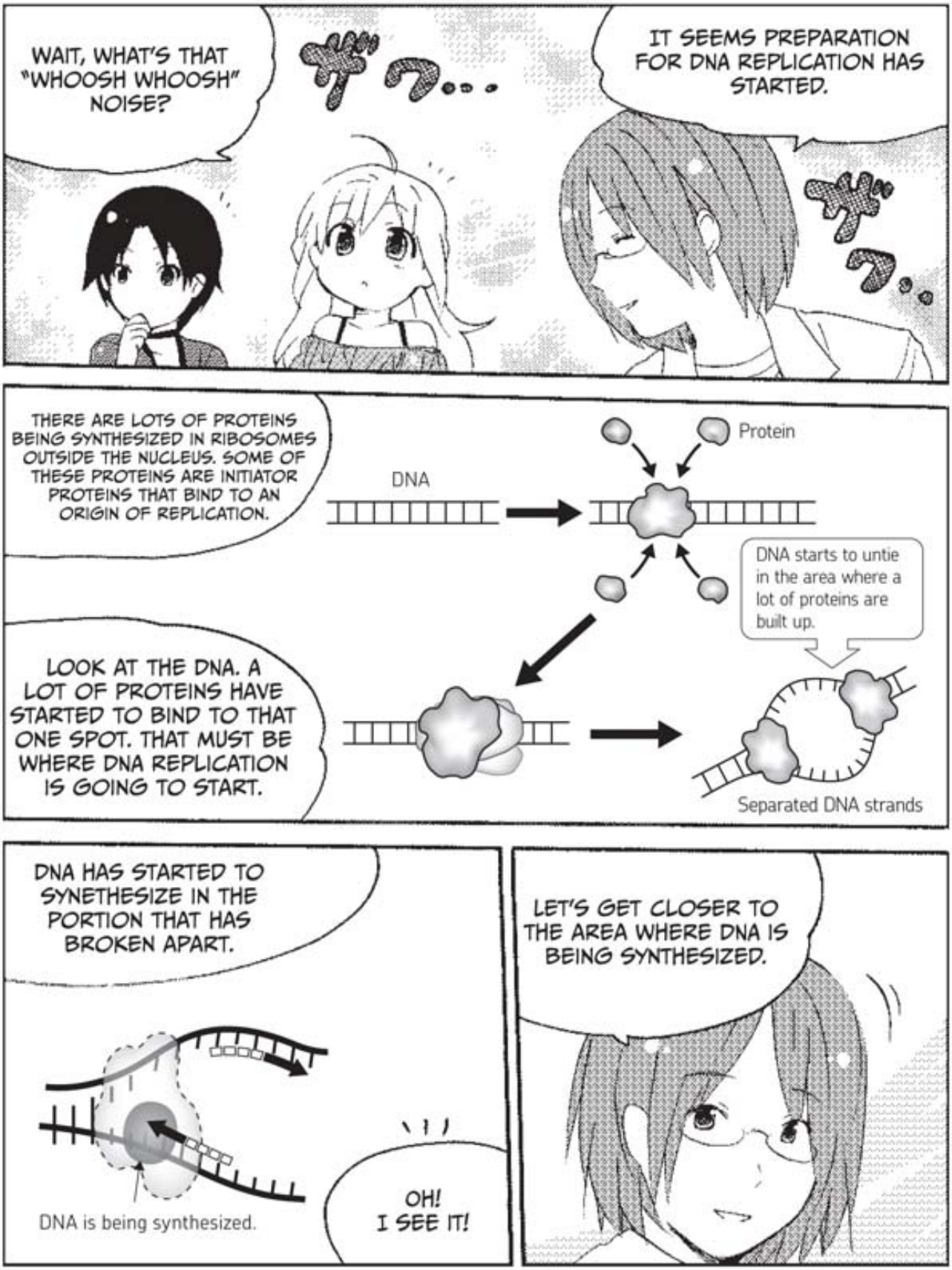

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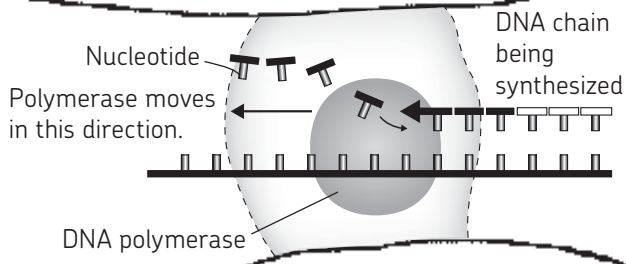
ONE OF THE PROTEINS BINDS TO THE NOW SINGLE-STRANDED DNA. NUCLEOTIDES, THE MATERIAL THAT MAKES UP DNA, ARE FLOATING AROUND, AND ATTACH TO THE SINGLE-STRANDED DNA.



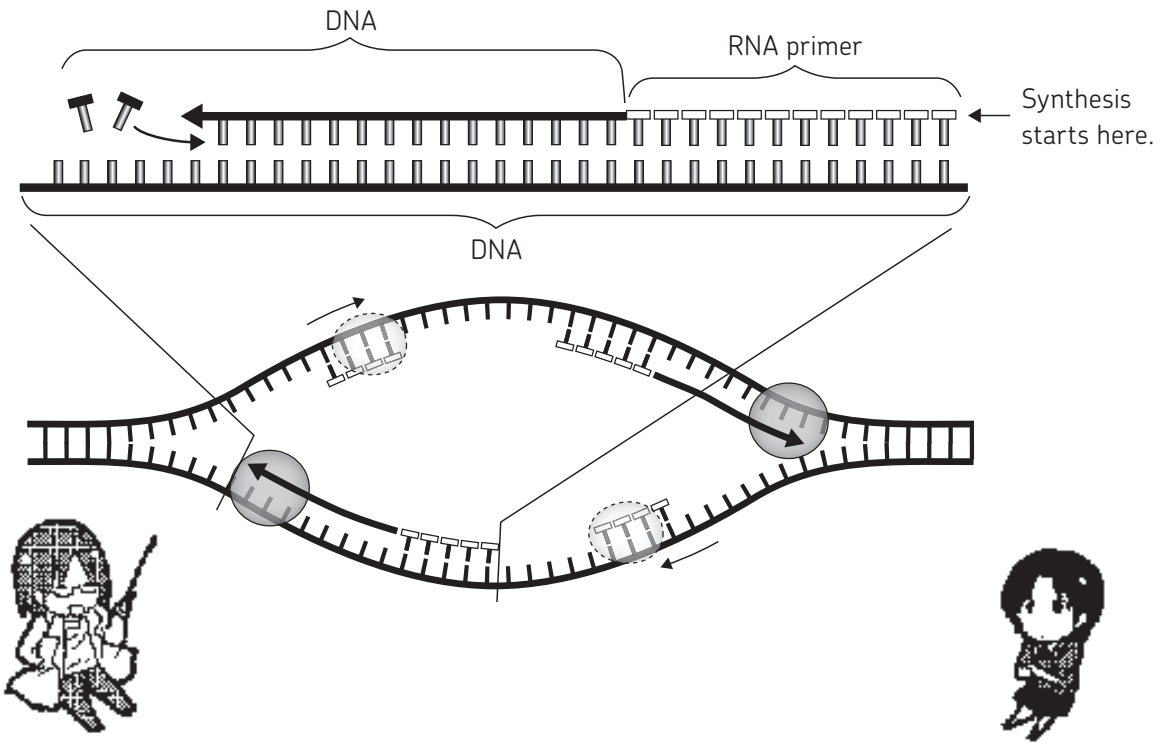
THAT PROTEIN IS THE DNA POLYMERASE.

LOOK CAREFULLY! DNA POLYMERASE DID NOT SYNTHESIZE DNA FIRST.

OH!

I SEE!

SYNTHESIS OF DNA ACTUALLY STARTS WHEN A SHORT STRAND OF RNA IS SYNTHESIZED. THIS SHORT STRAND OF RNA IS CALLED AN *RNA PRIMER*.



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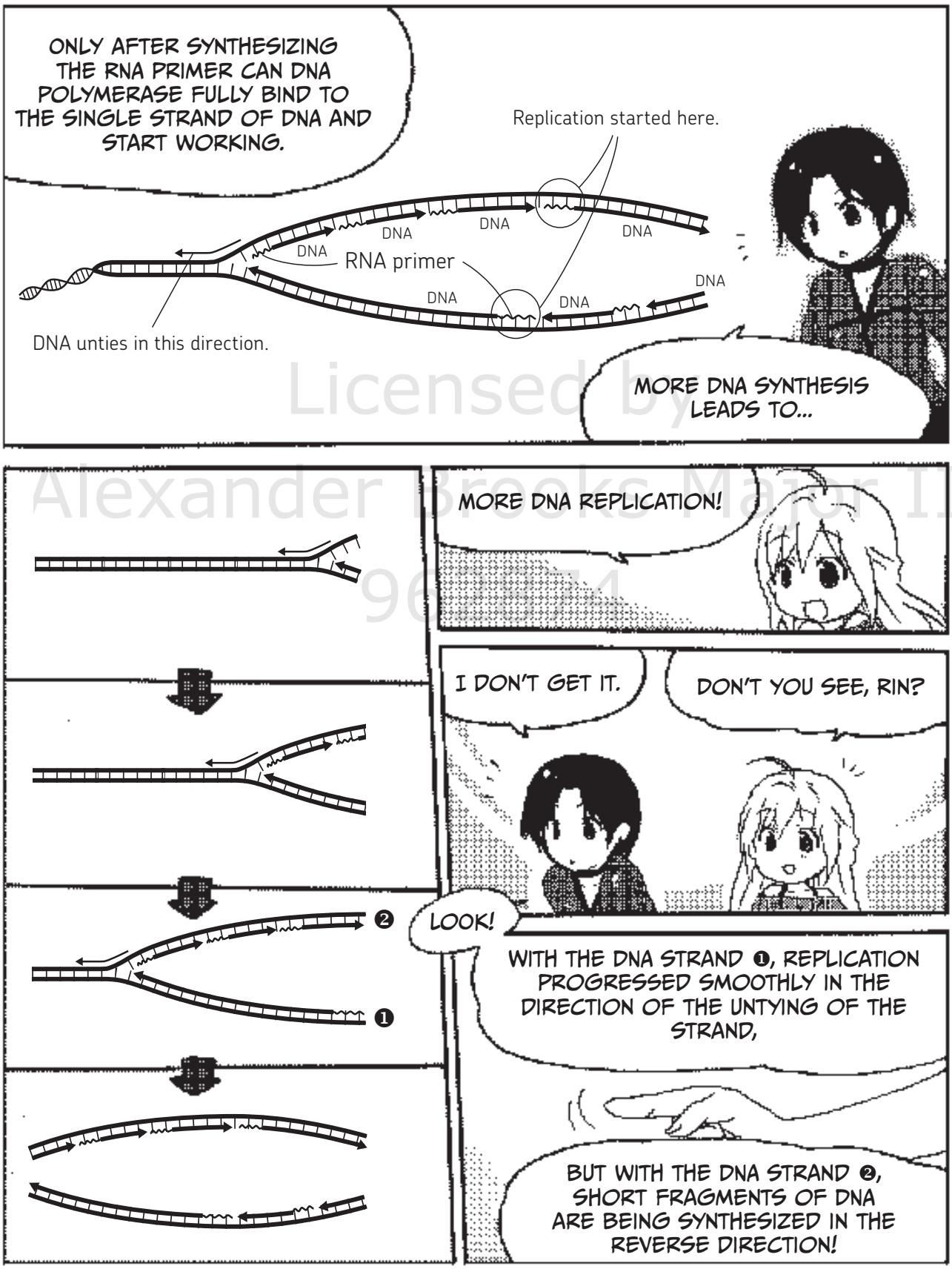

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Structure of a nucleotide

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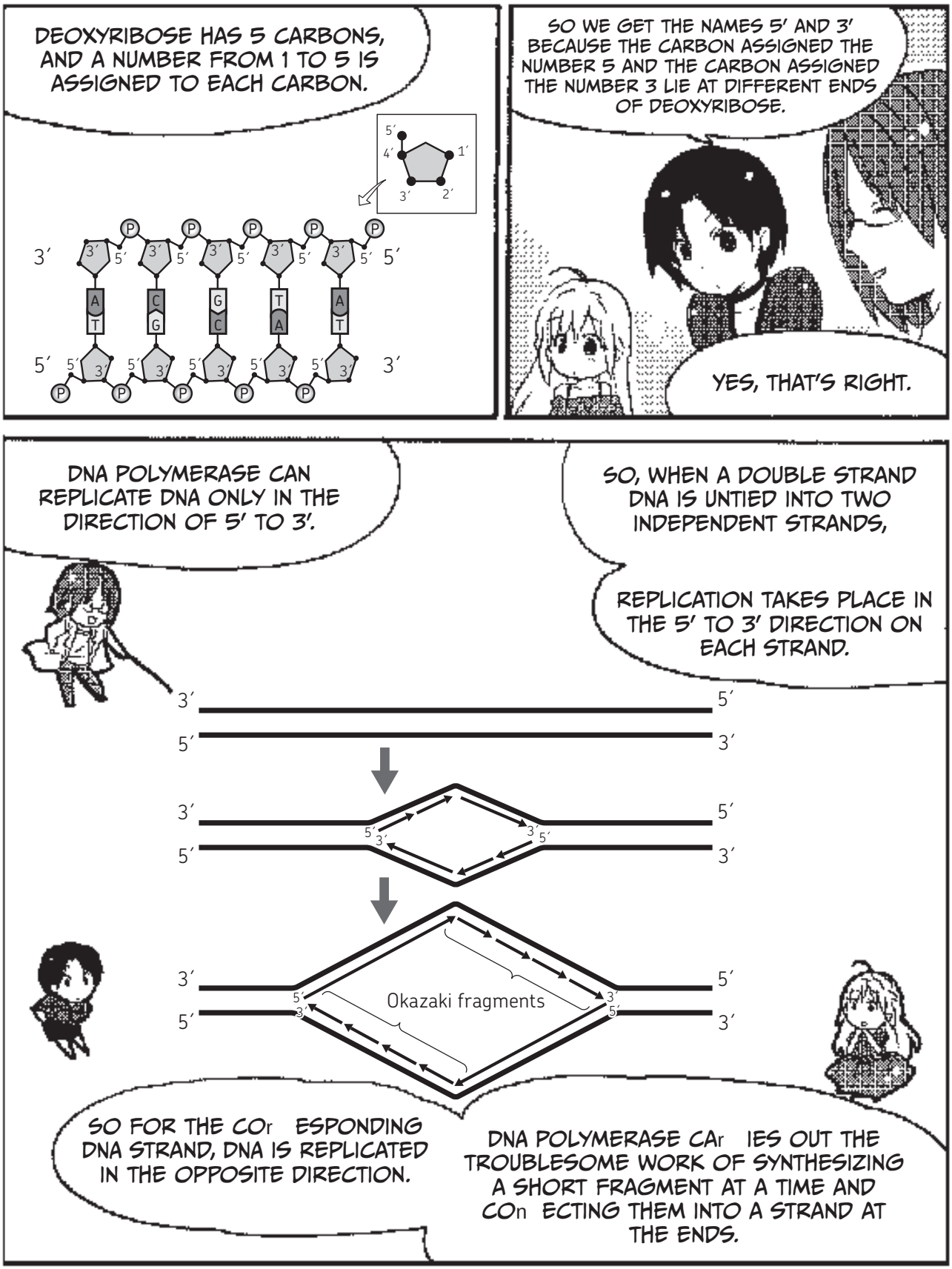

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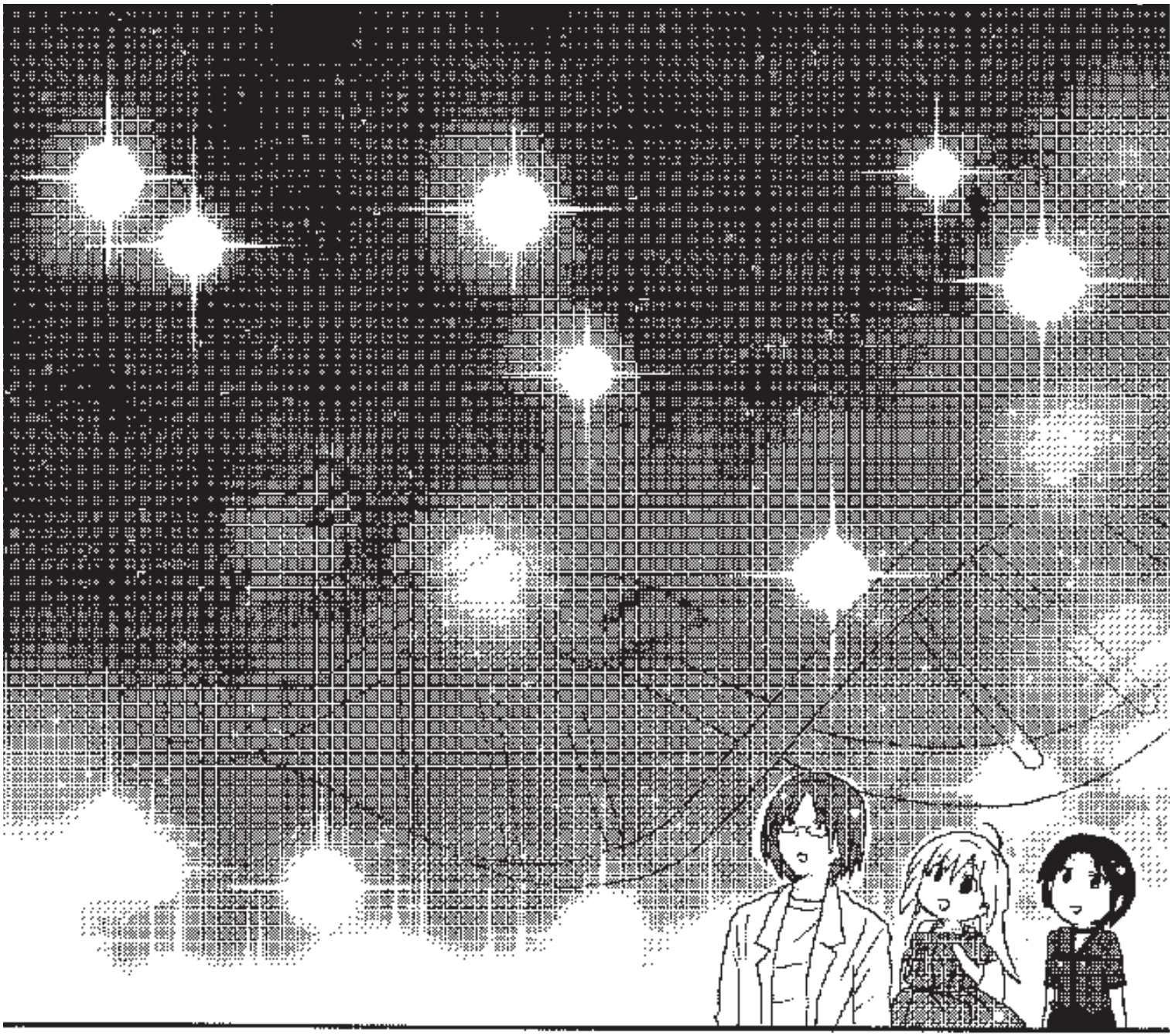

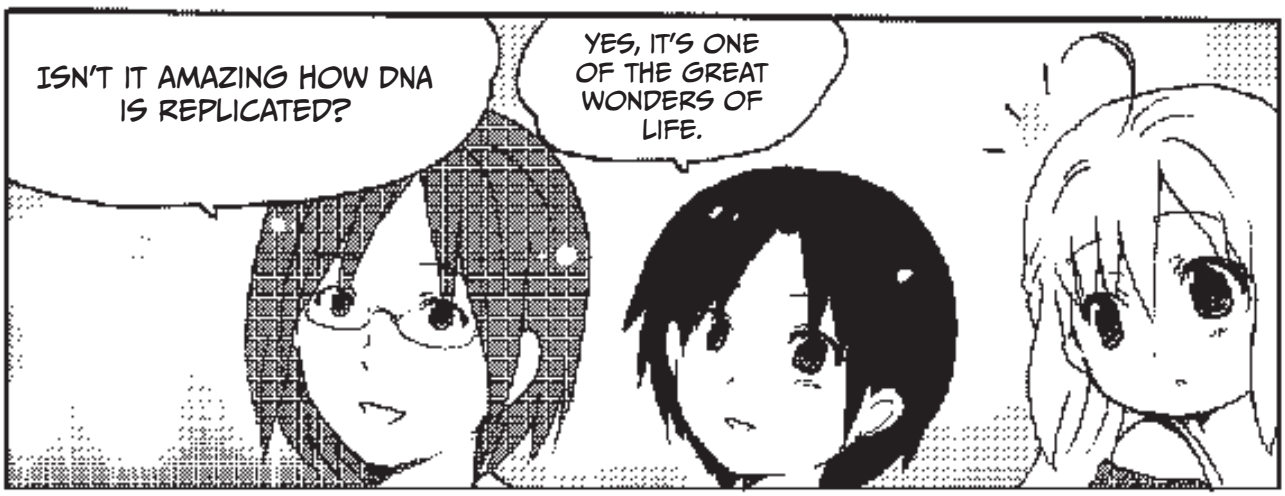

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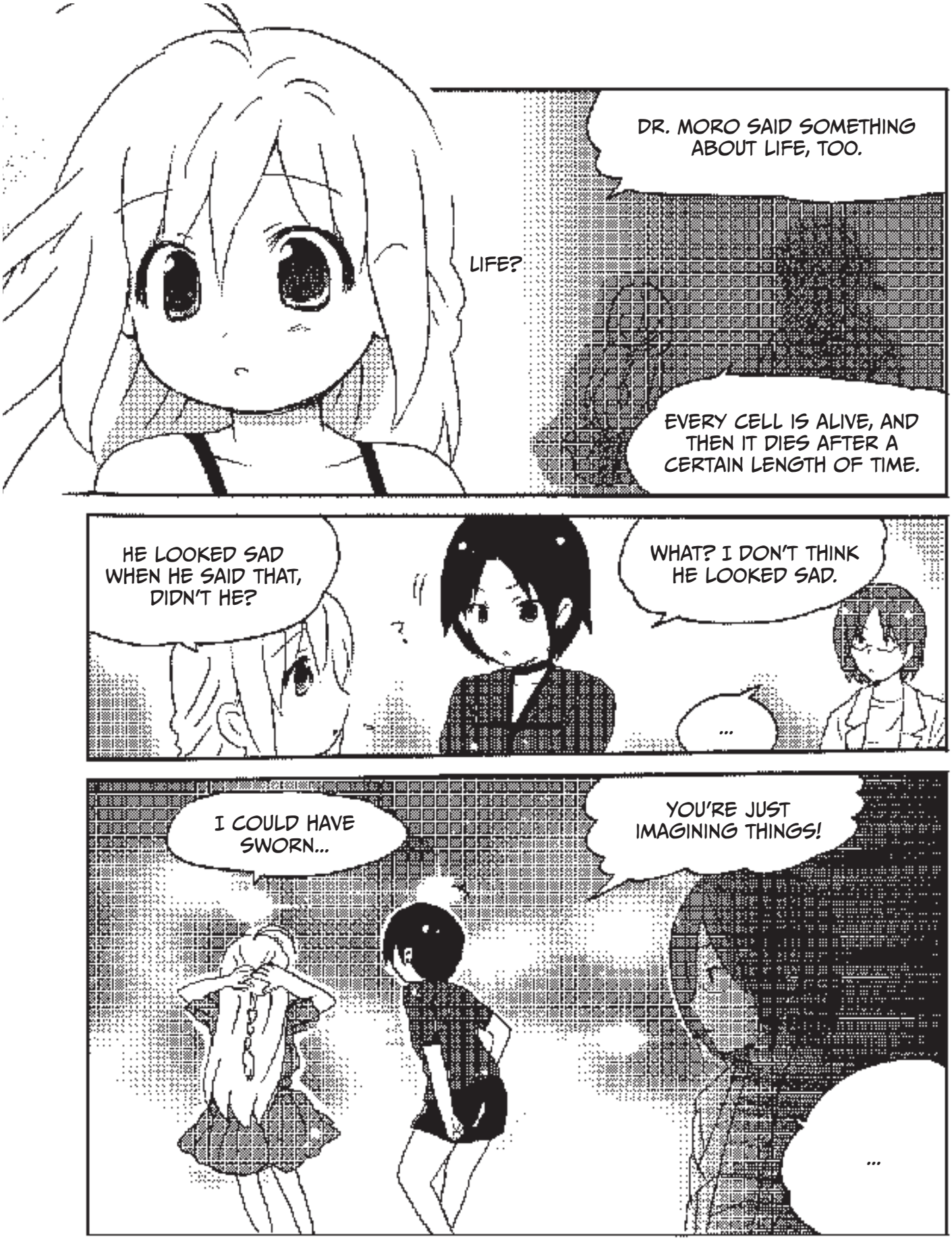

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WHAT IS A CHROMOSOME?

When we talk about cell division, we cannot avoid talking about chromosomes.

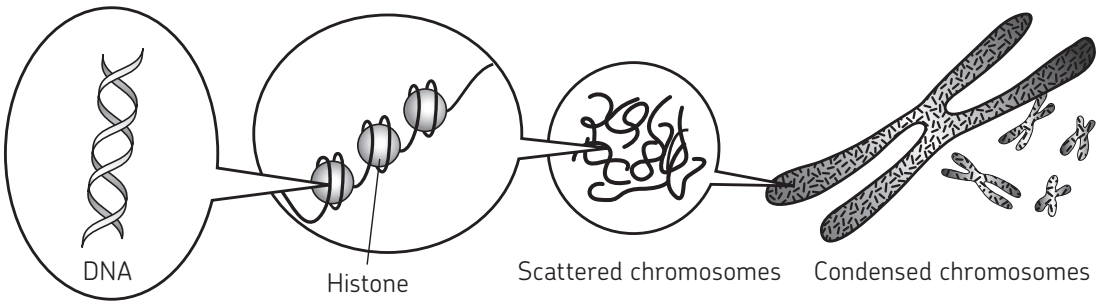
What's a chromosome?

A *chromosome* is DNA in a specific form—it contains genetic information. Before cell division starts, chromosomes gather together at the center of a cell. When cell division begins, chromosomes are torn apart into two pieces.

A chromosome is made up of one long string of a substance called *chromatin*. Do you remember the beaded chain that you learned about in Chapter 1? That is chromatin. It is made up of proteins called *histones*, DNA, and a few other molecules. Each bead in the chain is made up of 1.7 turns of DNA wound around a histone. A single strand of DNA winds around bead after bead, forming the thick thread of *chromatin*.

A histone is actually a set of proteins, eight molecules bound together, with two molecules of each of the following four types: H2A, H2B, H3, and H4.

Chromosomes, each a long strand of chromatin, are normally scattered throughout the nucleus and are invisible even with a microscope. Only when they condense into a compact shape for cell division do they become visible under the microscope.



DNA and chromosomes

Chromosomes, which were discovered in the 19th century, are named after their tendency to stain easily with certain dyes (*chroma* means *color* in Greek—you've probably seen it in other words like chromatic and monochrome).

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THE HUMAN BODY CONTAINS 24 CHROMOSOMES

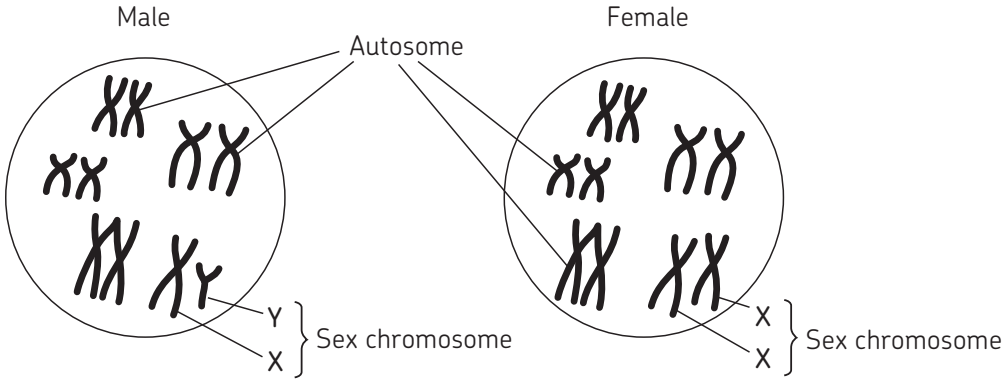
All human cells (except sex cells) contain 24 chromosomes.

But the number of chromosomes varies among living organisms. Higher order animals do not necessarily have more chromosomes than lower order animals. For example, a goldfish has about 100 chromosomes!

22 out of the 24 types are *autosomes* and are unrelated to gender. Each cell has two copies of each autosome. Why two? Because one is inherited from the father and the other from the mother.

A number from 1 to 22 is assigned to each autosome, with the largest chromosome being number 1. They are referred to as chromosome 3, chromosome 16, and so on. The two chromosomes which are not autosomes are *sex chromosomes*. They consist of an X chromosome and a Y chromosome.

These chromosomes determine the sex of a human being. Male cells have an X chromosome and a Y chromosome (XY), while female cells have two X chromosomes (XX).



CHROMOSOMES ARE ONLY VISIBLE AT THE TIME OF CELL DIVISION

Since chromosomes only thicken when replicated DNA and histones have completely condensed, they appear only when cells divide.

When cutting a large cloth into two pieces, for example, you can save time by folding it a few times before cutting it. In the same way, it is easier for cells to divide if DNA is condensed rather than dispersed inside the nucleus.

Now let's look at cell division.

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DYNAMIC CELL DIVISION

DNA is now replicated. The next phase starts.

As replication of DNA is completed, a cell begins to prepare to divide itself entirely into two. Cell division takes place in two general steps: mitosis and cytokinesis.

MITOSIS

Cell division starts in the nucleus, where DNA is contained. The process of dividing the contents of the cell's nucleus into two is called *mitosis*.

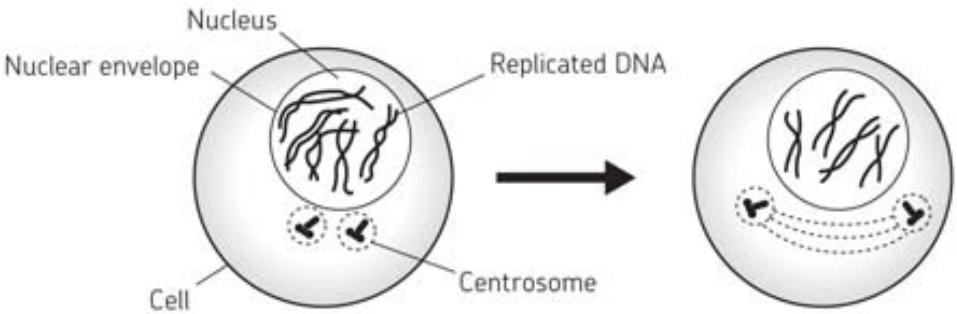
Is the division of a nucleus similar to atomic fission, which generates radioactivity and is used for atomic power generation?

All that happens inside the body?!

NOOOooooo!!! The nucleus of a cell is not the same thing as the nucleus of an atom. They have the same name, but they are very, very different things. Mitosis has nothing to do with nuclear fission!

Well, that's a relief!

In mitosis, replicated DNA is condensed tightly within the chromosomes, and the the chromosomes start to form a thick, condensed *X* shape. Substances called *centrosomes*, which used to be on the sides of the nucleus, start moving toward both poles of the cell.



Prophase

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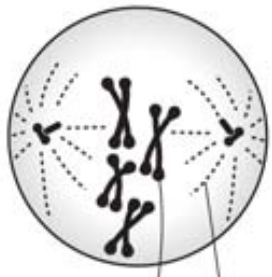
Although the process eventually creates two nuclei, the nucleus isn't just split in two. The original nuclear envelope is actually broken apart at the beginning of mitosis and then reformed after the cell divides.



Look! Something is changing around the centrosome!

You're right. String-like substances called *spindle fibers* start extending from the centrosomes, each of which is now located on opposite poles. These spindle fibers are made up of long thin substances called *microtubules*.

After the membrane has disappeared, replicated DNA that has started to condense is flung out to the sea of cytoplasm. This process is important.

After the condensation of chromosomes has finished and the formation of their shape is almost completed, the string-like spindle fiber being extended from the centrosomes on each pole reaches near the center of each chromosome and sticks tight there. The existence of the nuclear envelope would be a hindrance to the spindle fiber if it hadn't disappeared.

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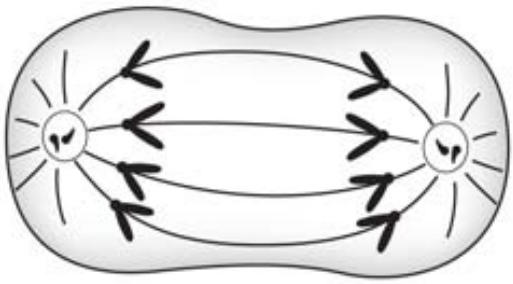
The spindle fibers move the chromosomes to the middle of the cell. This is called the *mitotic spindle* or *spindle apparatus*.

Anaphase



During anaphase, the spindle fibers stuck to each chromosome pull toward both poles of the cell. As a result, the chromosomes being arranged at the center are drawn apart to each pole.

Some time after being drawn apart, the chromosomes start to scatter and unfold to restore their original state. They become invisible even with a microscope. Then formation of the nuclear envelope, currently scattered in the cytoplasm, starts, and the shape of a nucleus begins appearing in each pole.

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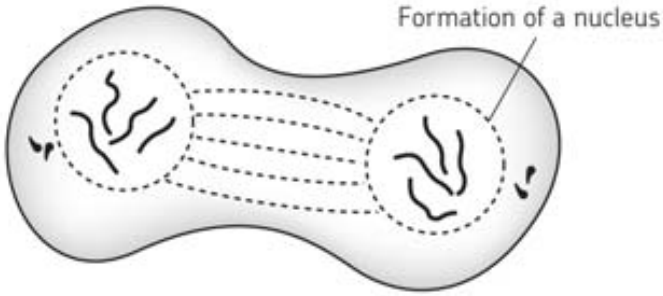
Telophase



In order for cells to divide and survive, the DNA has to replicate and be divided evenly into two new nuclei rather than just haphazardly splitting the original nucleus in two.

Because of the appearance of the mitotic spindle, this step is referred to as *mitotic division*.

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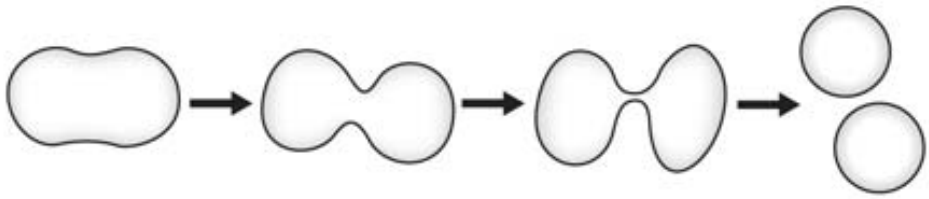
CYTOKINESIS

Is the process of division the same for animals and plants?

It is almost the same as far as mitosis is concerned. In cytokinesis, which occurs later, the entire cell starts to divide into two. There is a big difference between animal cells and plant cells in this process.

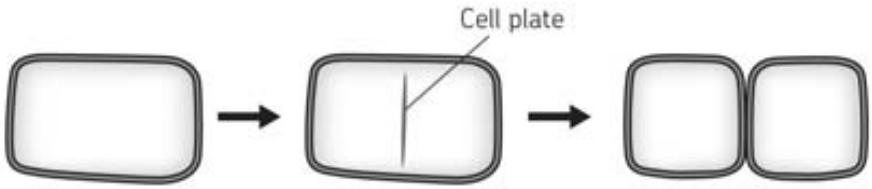
In animal cells, the portion near the center of a cell constricts, and this constriction progresses until the cell is divided equally into two. The important part about cytokinesis is that all of the organelles, cytoplasm, and nutrients in the cell are also divided evenly between the two new cells. It's just like making cookies; you're trying to split cookie dough into smaller pieces with an equal number of chocolate chips and nuts in each piece.

Animal cell



Meanwhile, since plant cells are surrounded by a hard substance called a *cell wall*, a different process takes place. In plant cells, a wall called a *cell plate* appears at the center of the cell. Then it gradually grows larger and larger until the cell is divided into two large portions, and the cell is divided in two.

Plant cell



In this way, a cell completes division, and DNA is safely contained in both cells.

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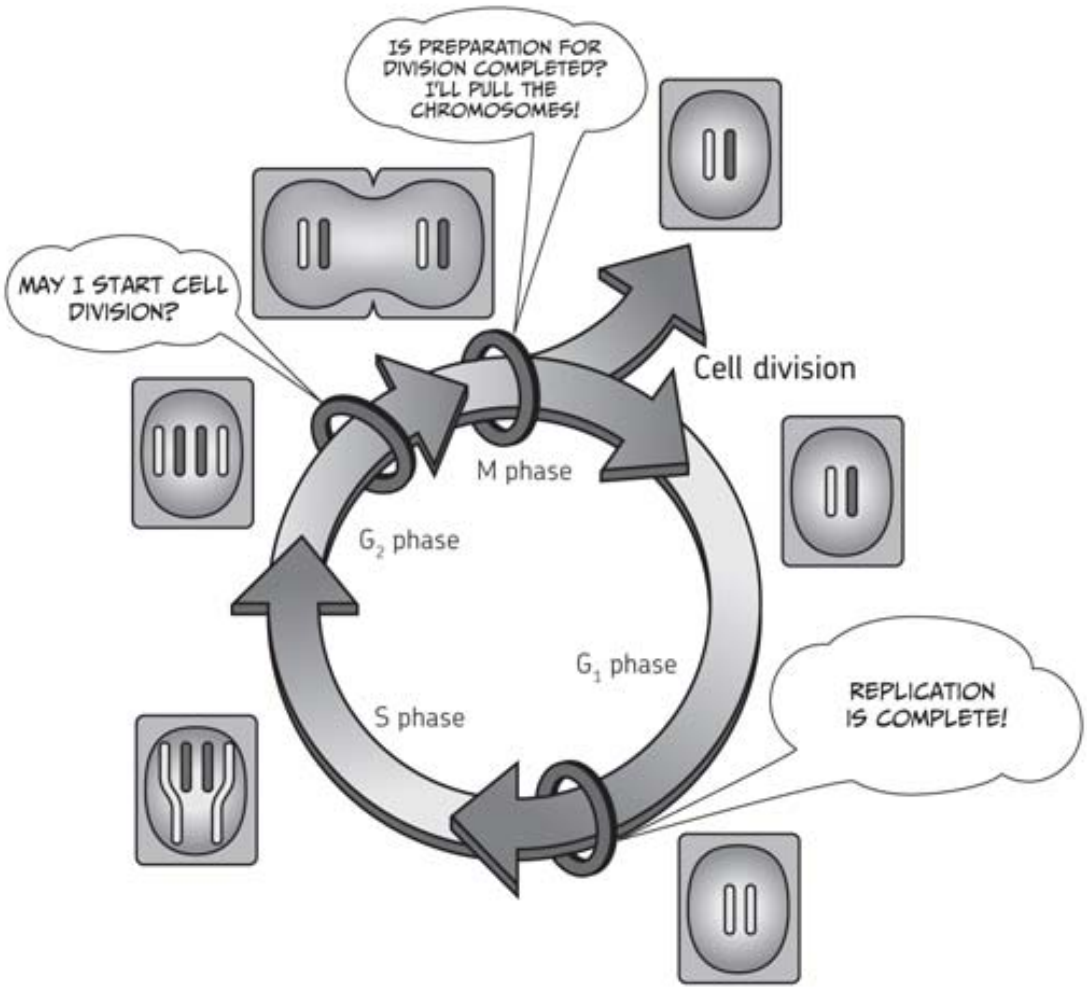
WHAT IS A CELL CYCLE?

Some cells continue dividing indefinitely and some do not. A cell's function will determine how often it replicates. Basal cells (see page 103), which you saw deep in the skin, are a type of cell that continues dividing endlessly, as skin cells are constantly replaced. Basal cells have limited lives. Older basal cells at the end of their lives are believed to stop dividing.

As you learned in this chapter, cell division follows predetermined steps: DNA is replicated, chromatin condenses into chromosomes, the nuclear envelope disappears, the cell's contents are split in two, and an entire cell divides.

A cell has many checkpoints during replication to make sure no errors occur during this important process.

This ring indicates a check point in the cell cycle.



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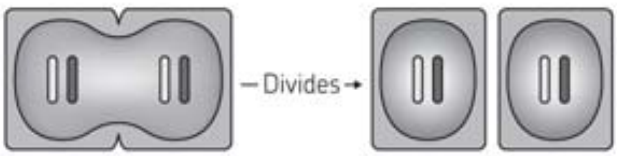
Cells that divide many times repeat these steps again and again. The series of steps in which a cell completes one division is called a *cell cycle*. The cell cycle is divided into four phases:

- **G_1 phase** The cell prepares for DNA replication in this step. Enzymes that are needed for DNA replication (the S phase) are produced at this time, along with other proteins the cell needs. You can think of G_1 as standing for the first growth stage of the cell.

- **S phase** DNA is replicated in this step. DNA is synthesized, so this step is called the S phase.

- **G_2 phase** The cell prepares for division in this step. Microtubules that are needed during the M phase are synthesized at this time. This is called G_2, as it's the second period of growth and protein synthesis. Collectively, the G_1, S, and G_2 phases are referred to as *interphase*. As you may expect, a cell's preparation for division (interphase) is a much longer process than cell division itself (the M phase).

- **M phase** Mitosis and cytokinesis are carried out in this step. It is called the M phase because this is when mitosis occurs.



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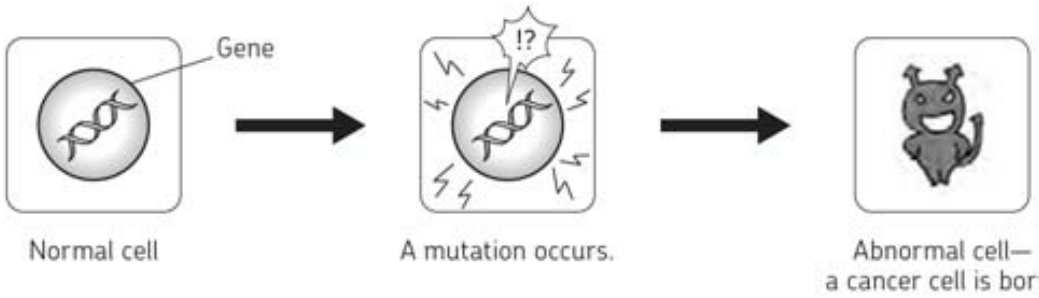
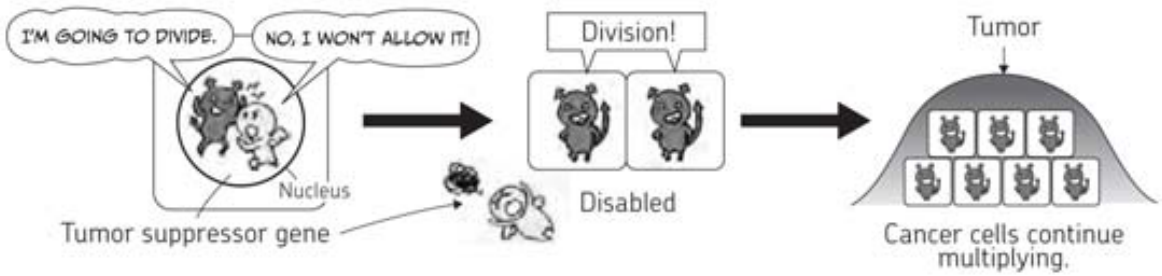
WHAT CAUSES CANCER?

A *cancer cell* is one that was once functioning normally but then suddenly gets a mutation in its genes that make the cell go "mad." These changes in its genes make cancer cells multiply without regard for other cells, growing out of control and stealing energy and nutrients from other cells in the tissue. If cancer cells continue multiplying, they can become visible under a microscope, or even to human eyes in the form of a tumor.

There are many ways a healthy cell can become a cancer cell, but no matter the reason, all cancer cells carry on multiplying because the functions of their genes that regulate cell division have gone out of control.



With normal cells, tumor suppressor genes act like a brake; these genes apply the break at the checkpoints in the cell cycle to prevent arbitrary replication of the cell. However, in some cells, these tumor suppressor genes become mutated and lose their ability to function. As a result, the brake is disabled, allowing the cell to divide over and over again.



In some cancer cells, genes that work to accelerate and promote cell division go awry, and the growth and division of the cell become *super accelerated*. The tumor suppressor genes cannot do their job if this happens. The cells will repeatedly divide to become cancerous and interfere with the normal functions of tissues and organs.

A multicellular organism is like a society made up of cells. The cells don't normally do things that disturb the order of the society. The cells divide as needed and carry out their special assignments. However, cancer cells are like gangs who steal and recruit new members, spreading through society and disrupting order.

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4

HOW IS A PROTEIN MADE?

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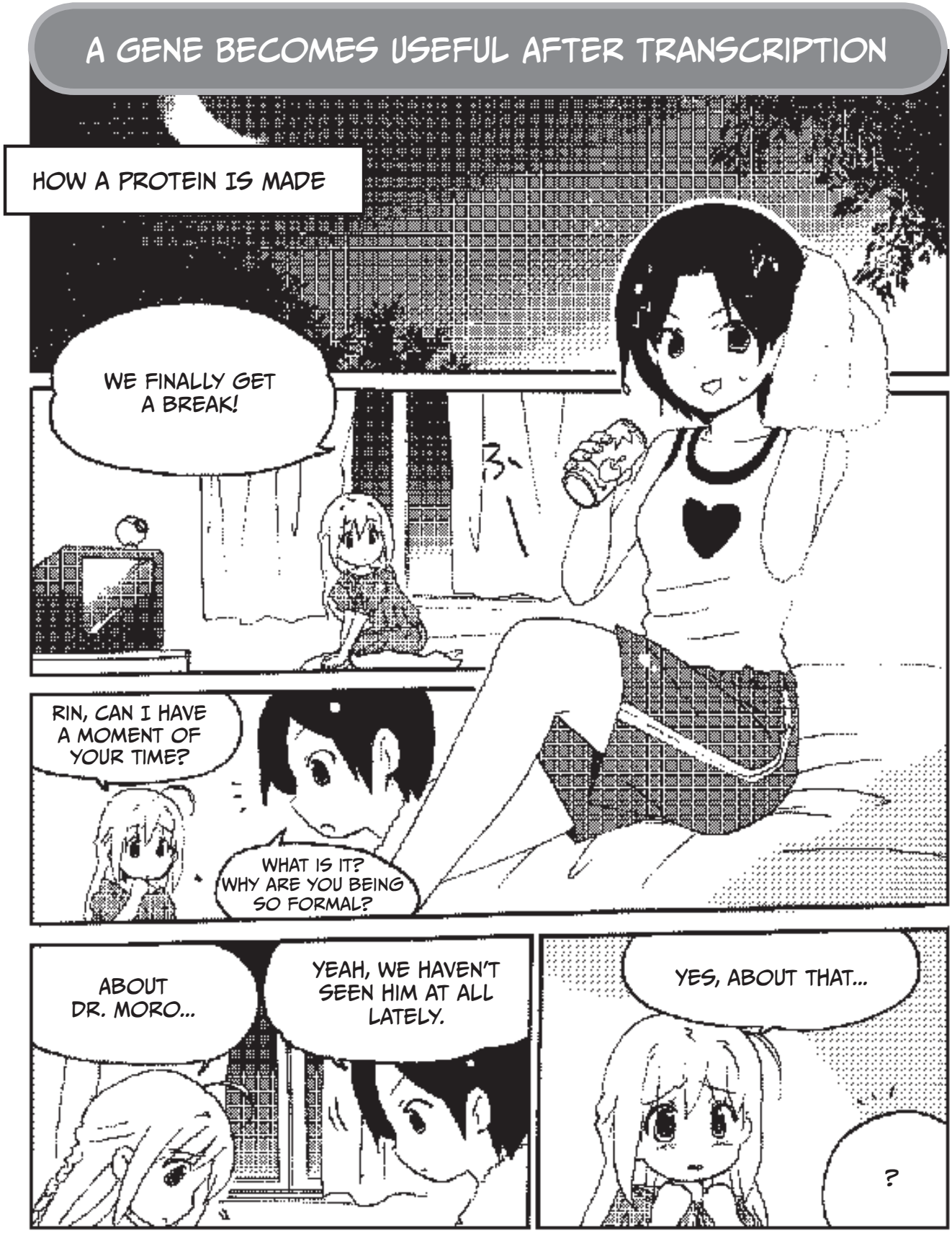
A GENE BECOMES USEFUL AFTER TRANSCRIPTION



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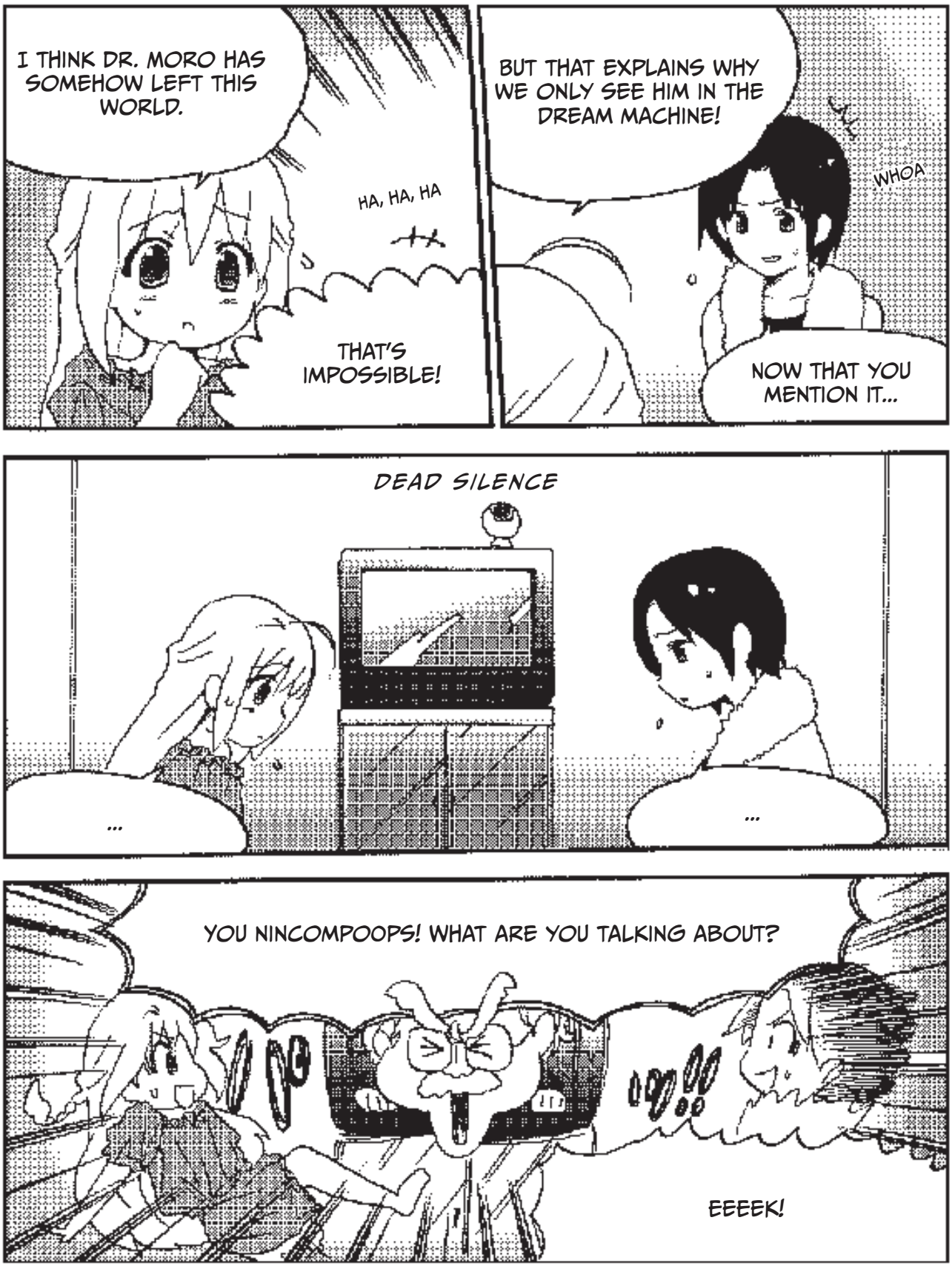

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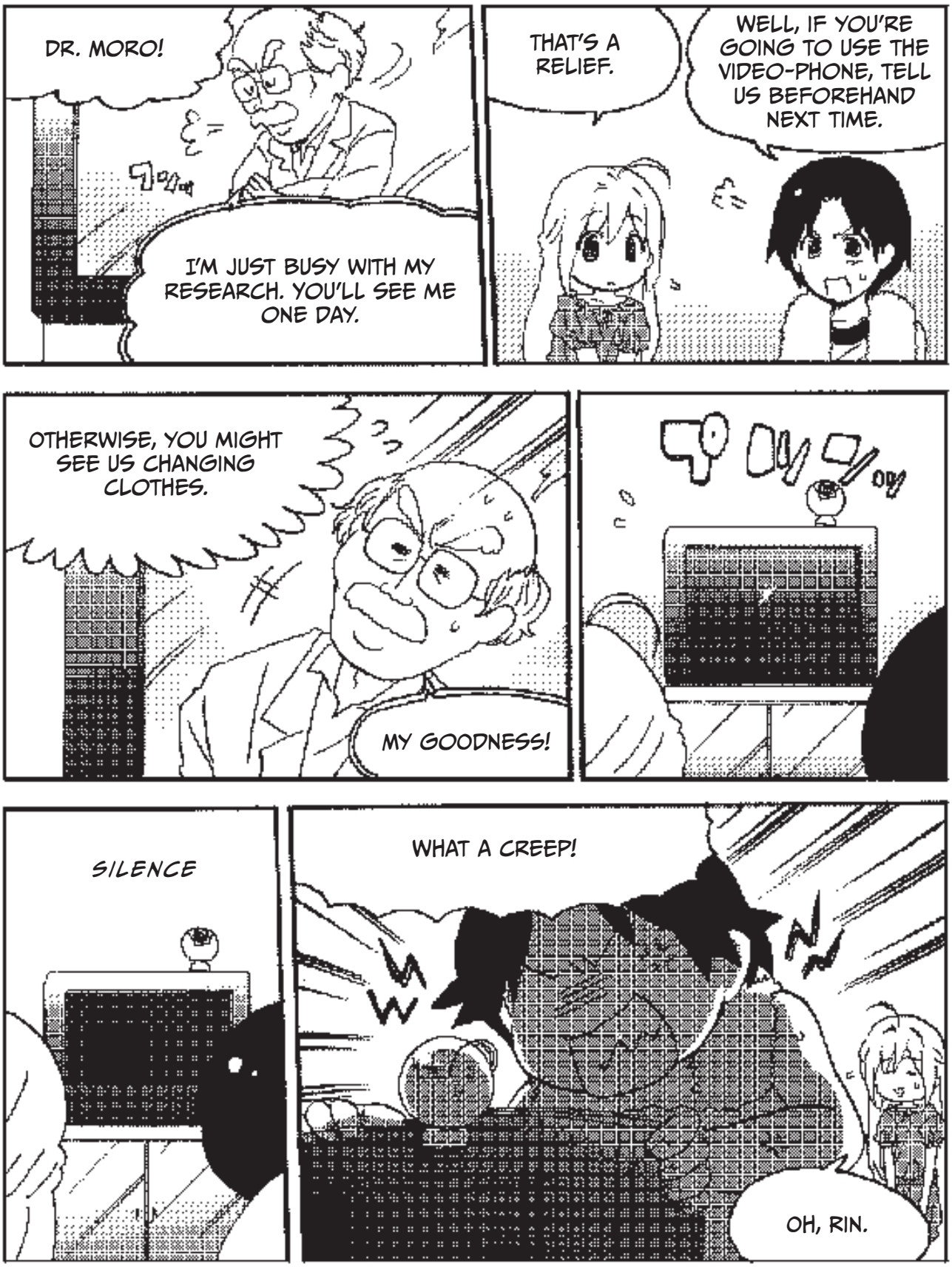

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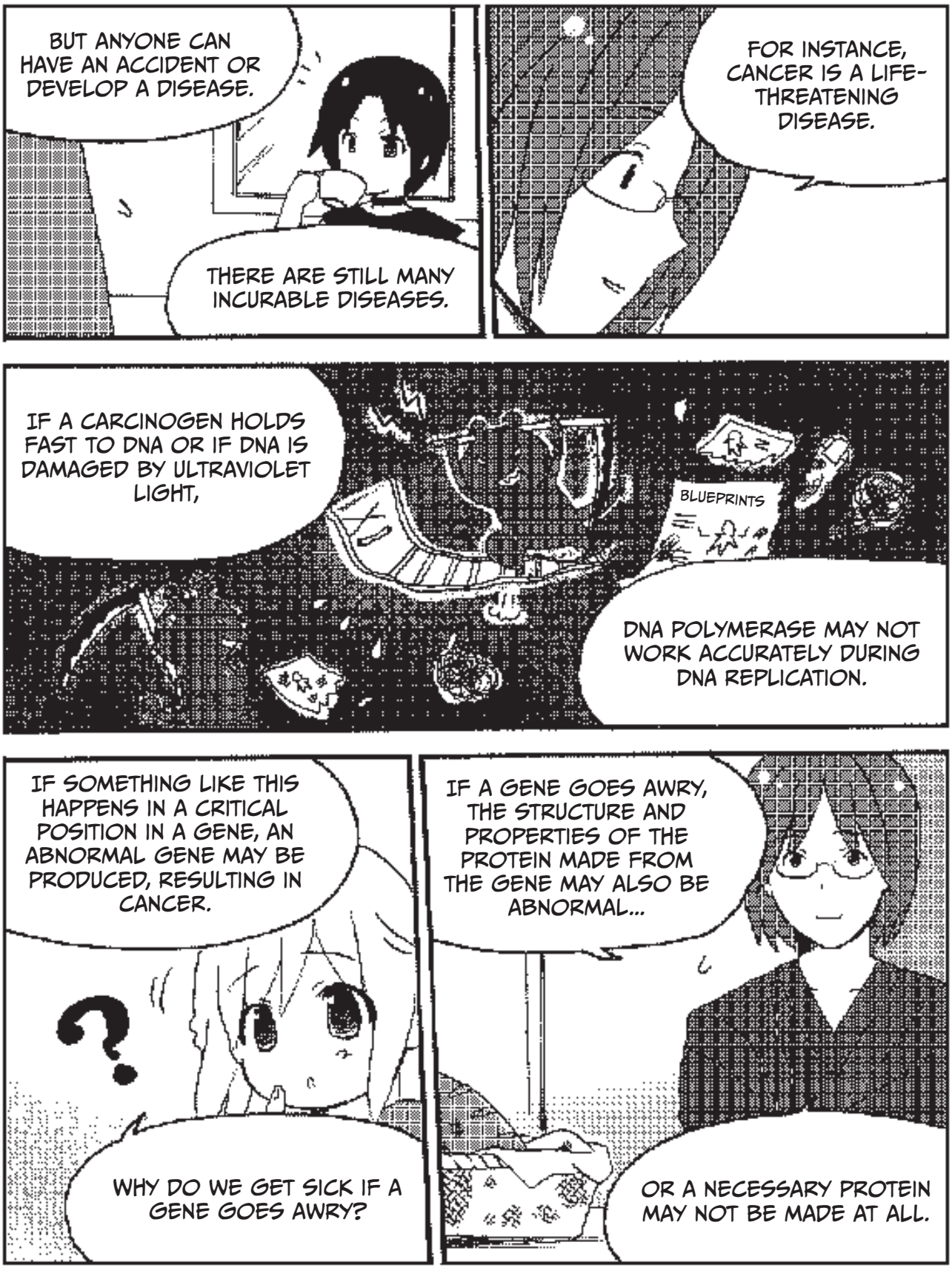

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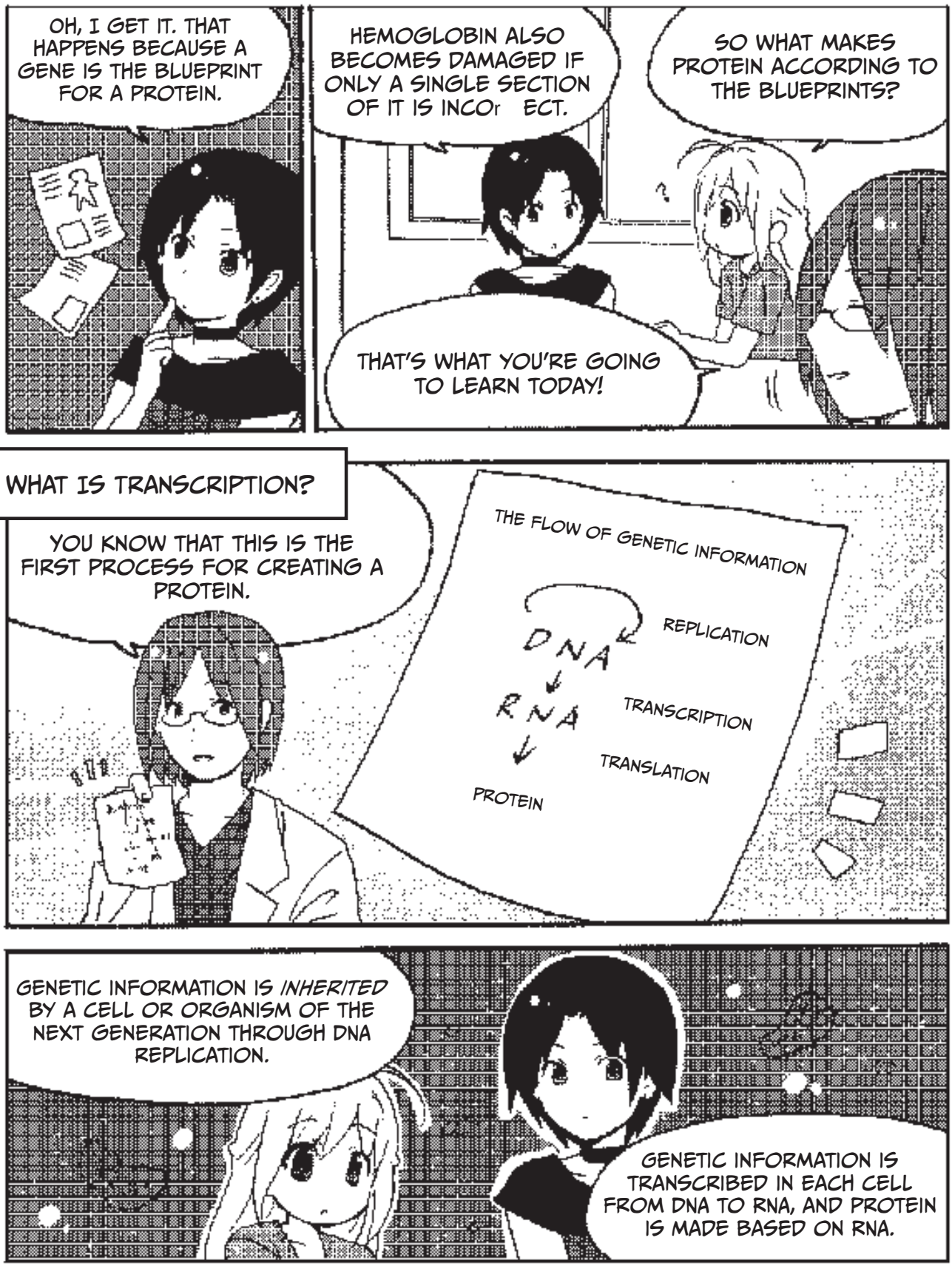

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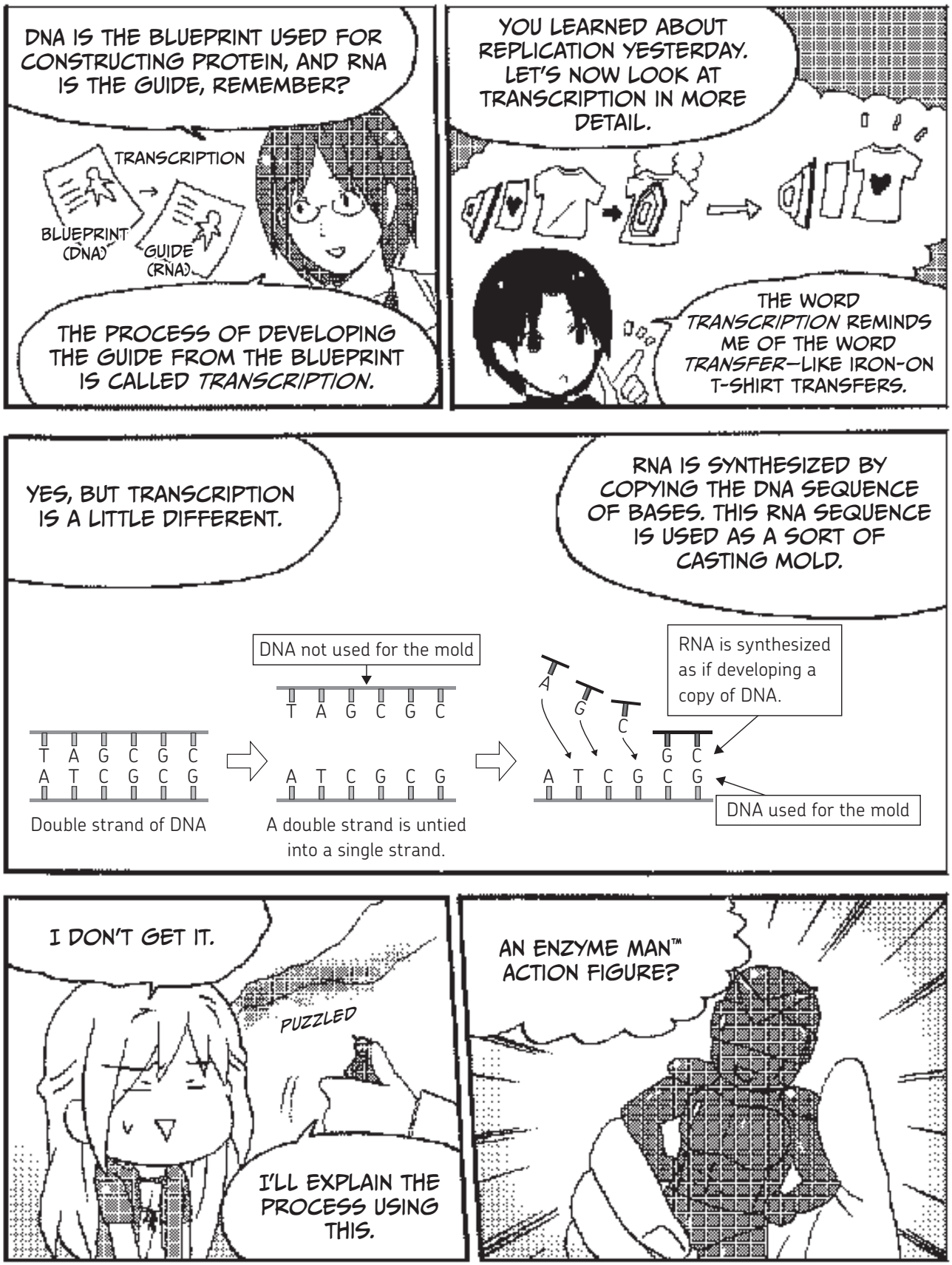

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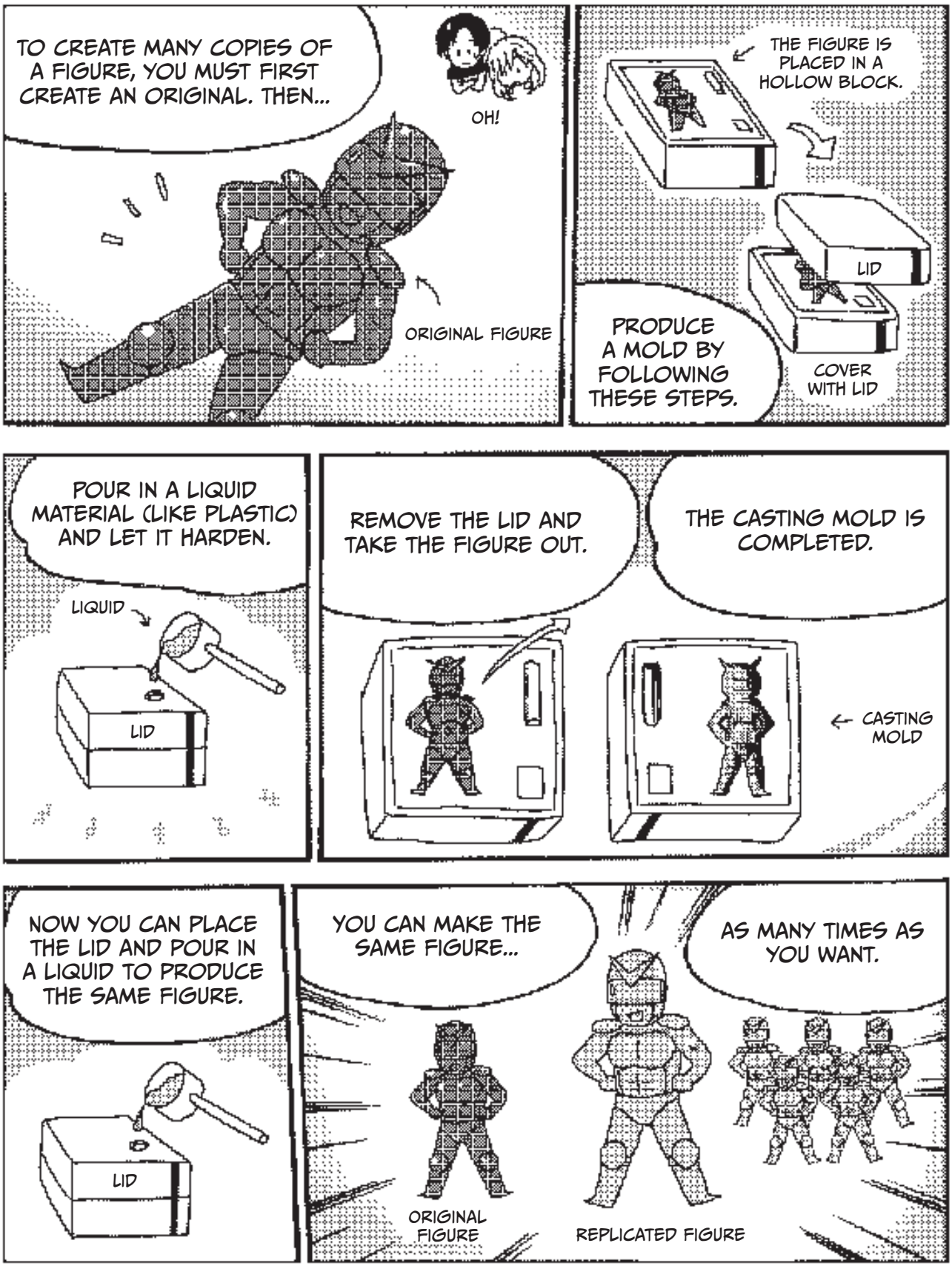

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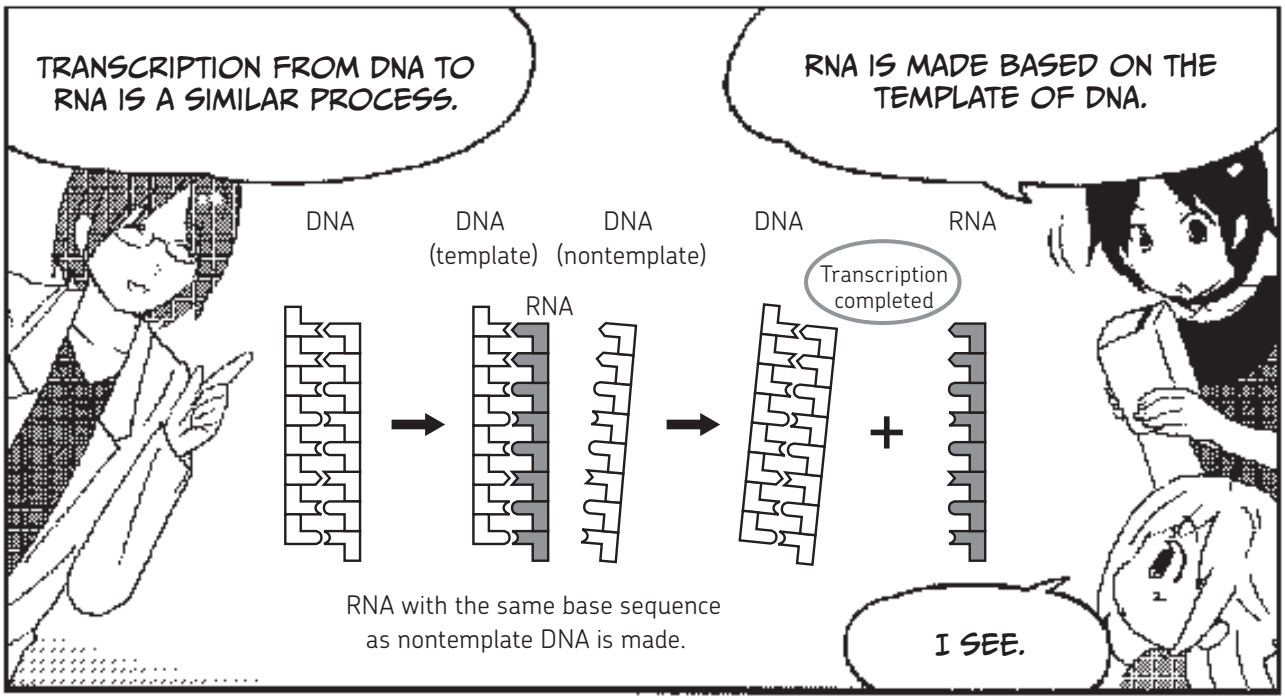

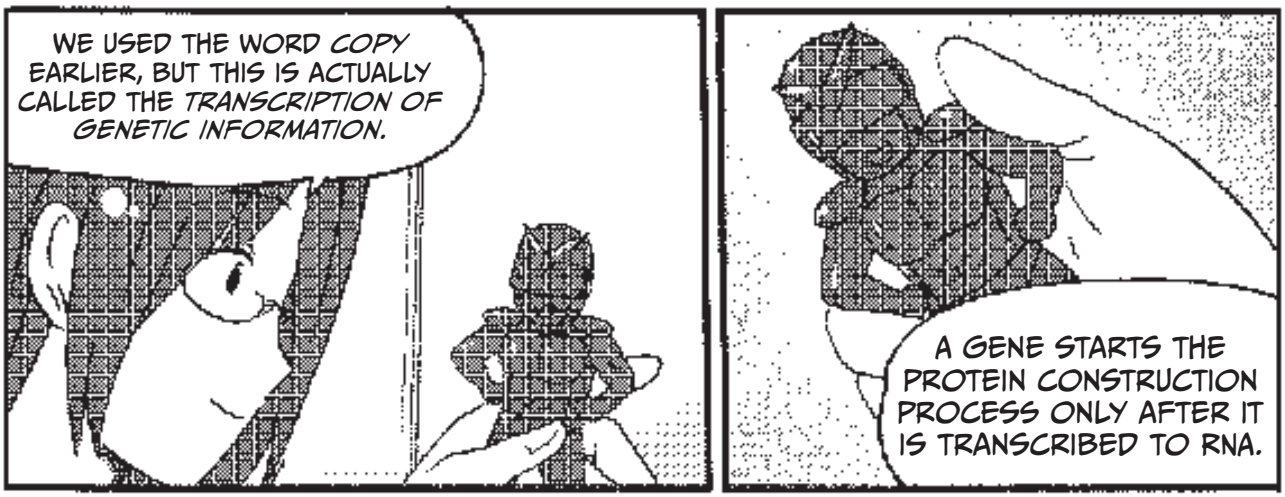

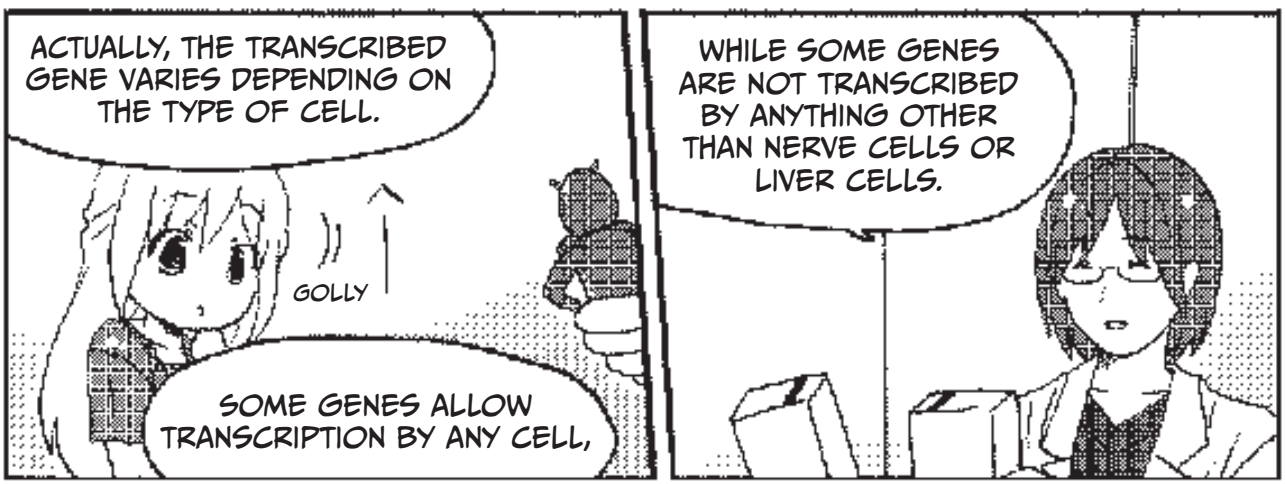

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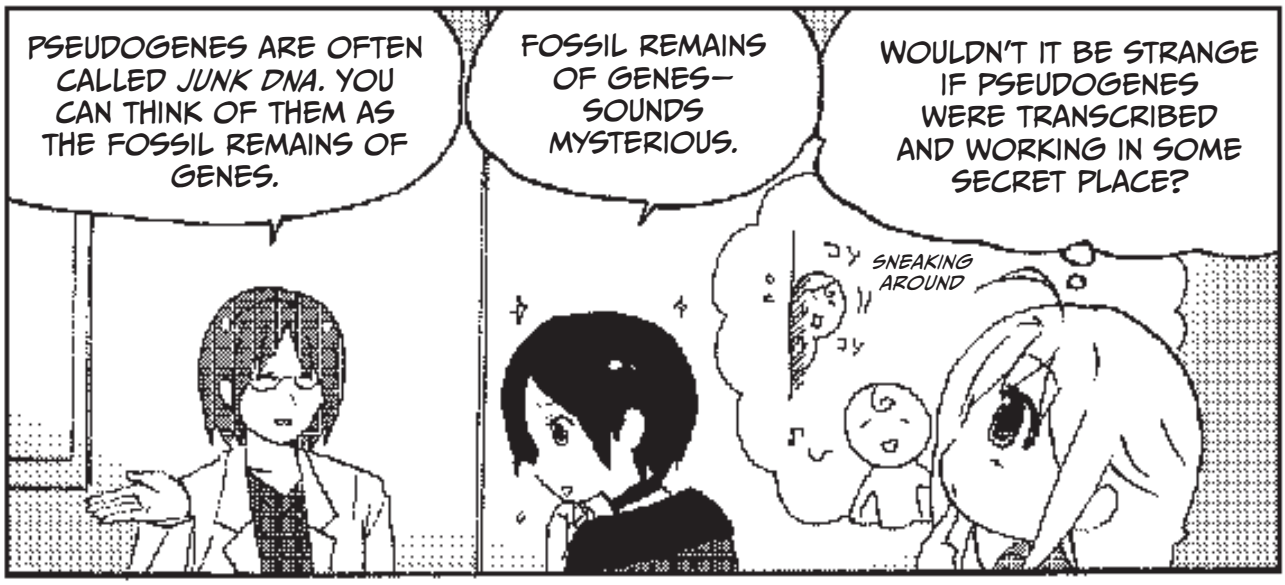

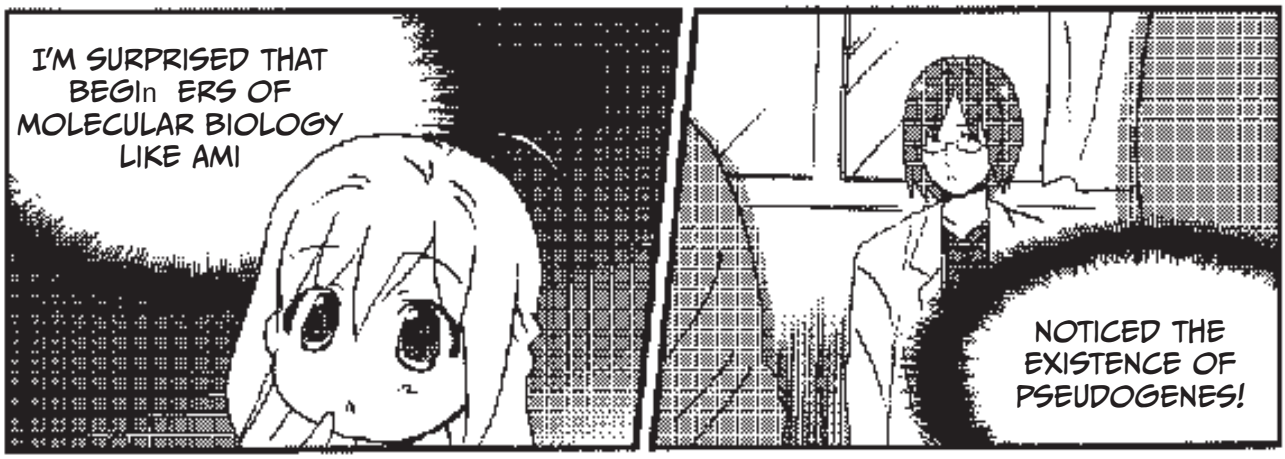

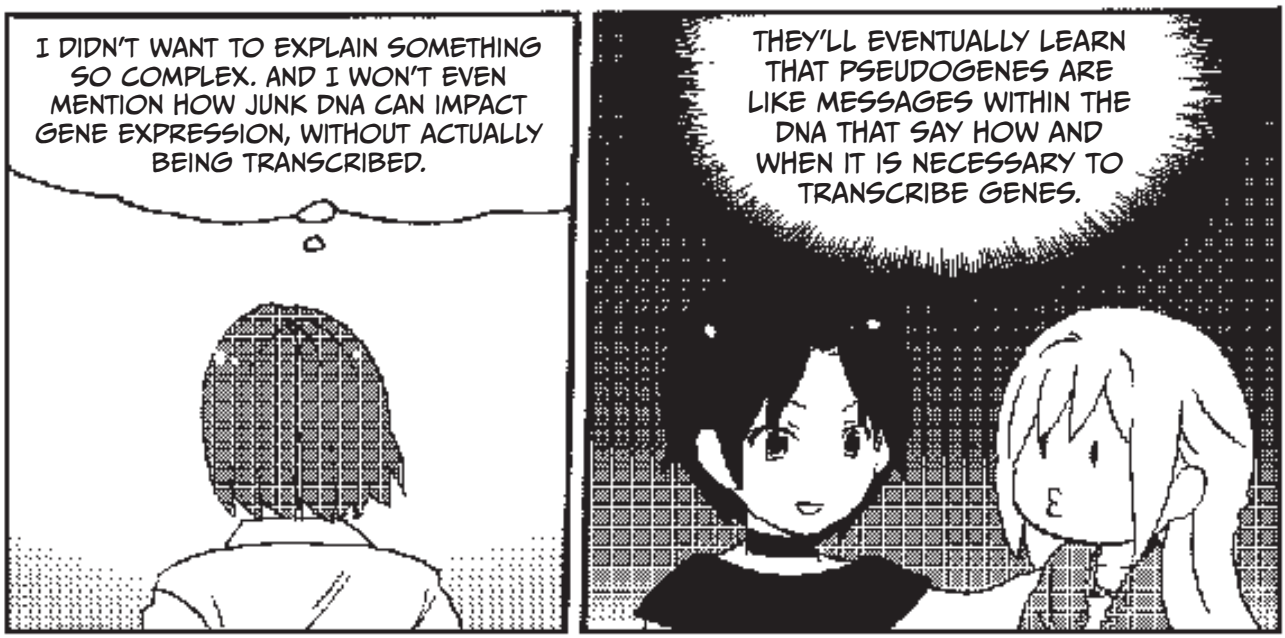

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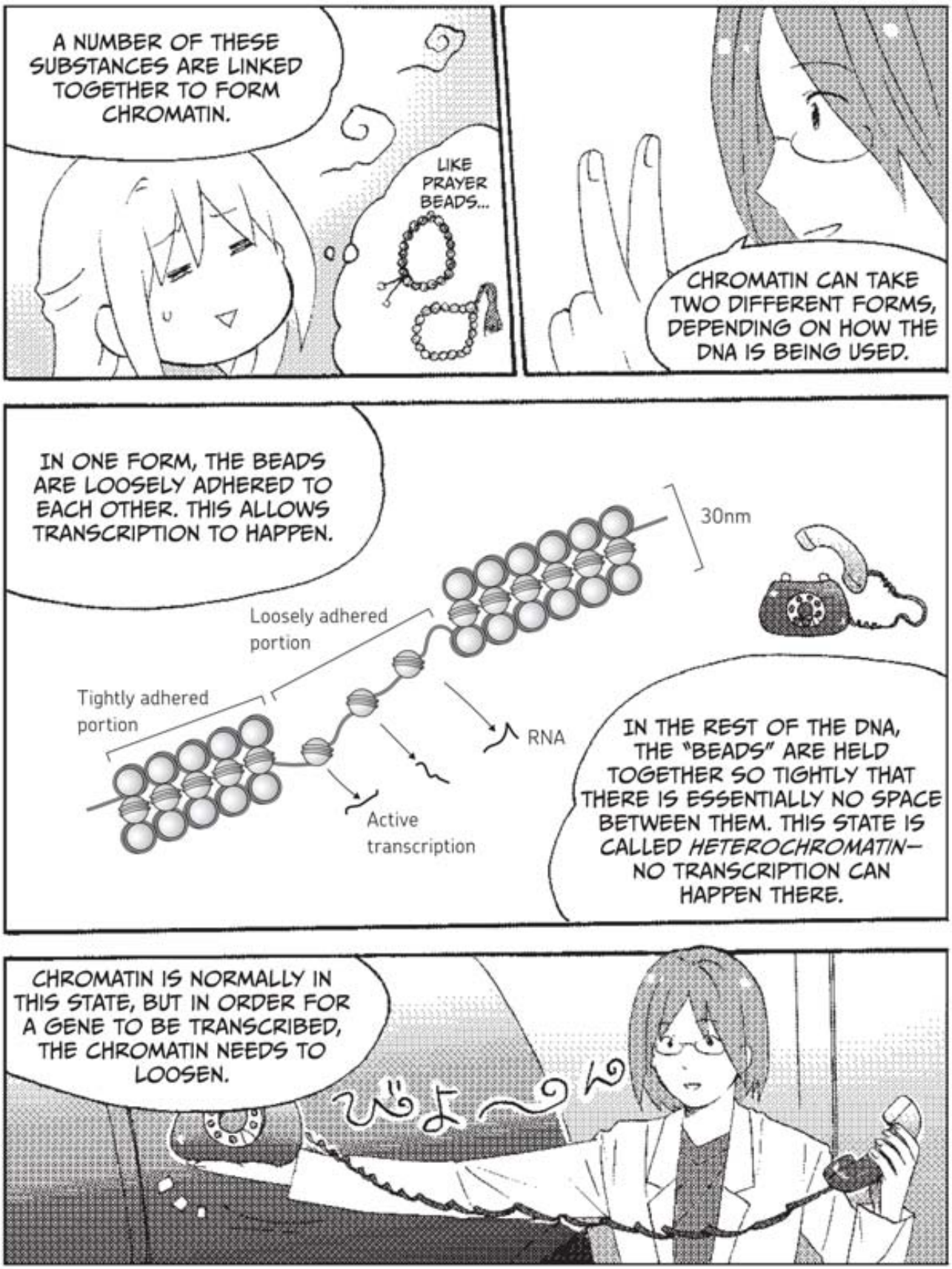

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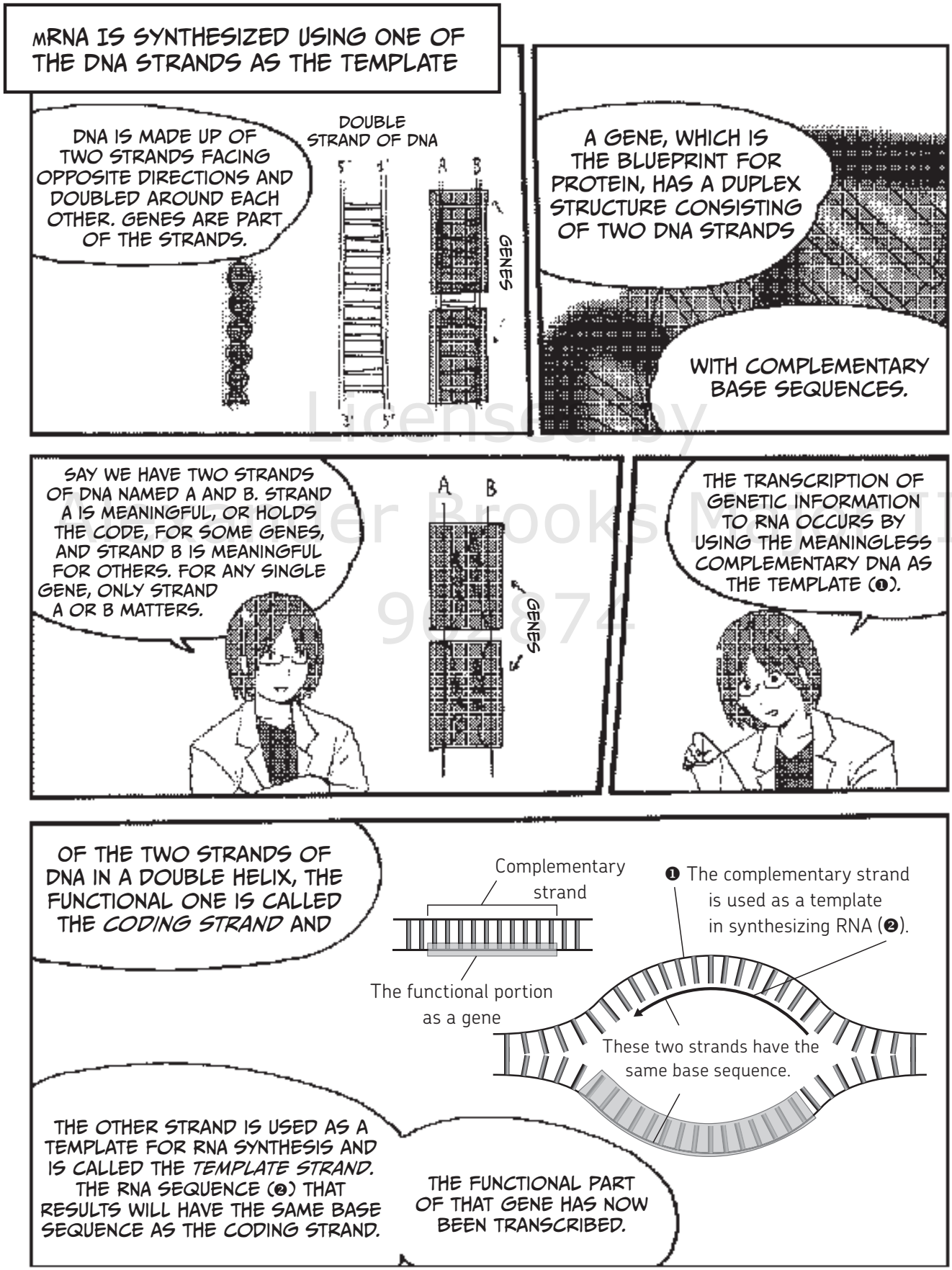

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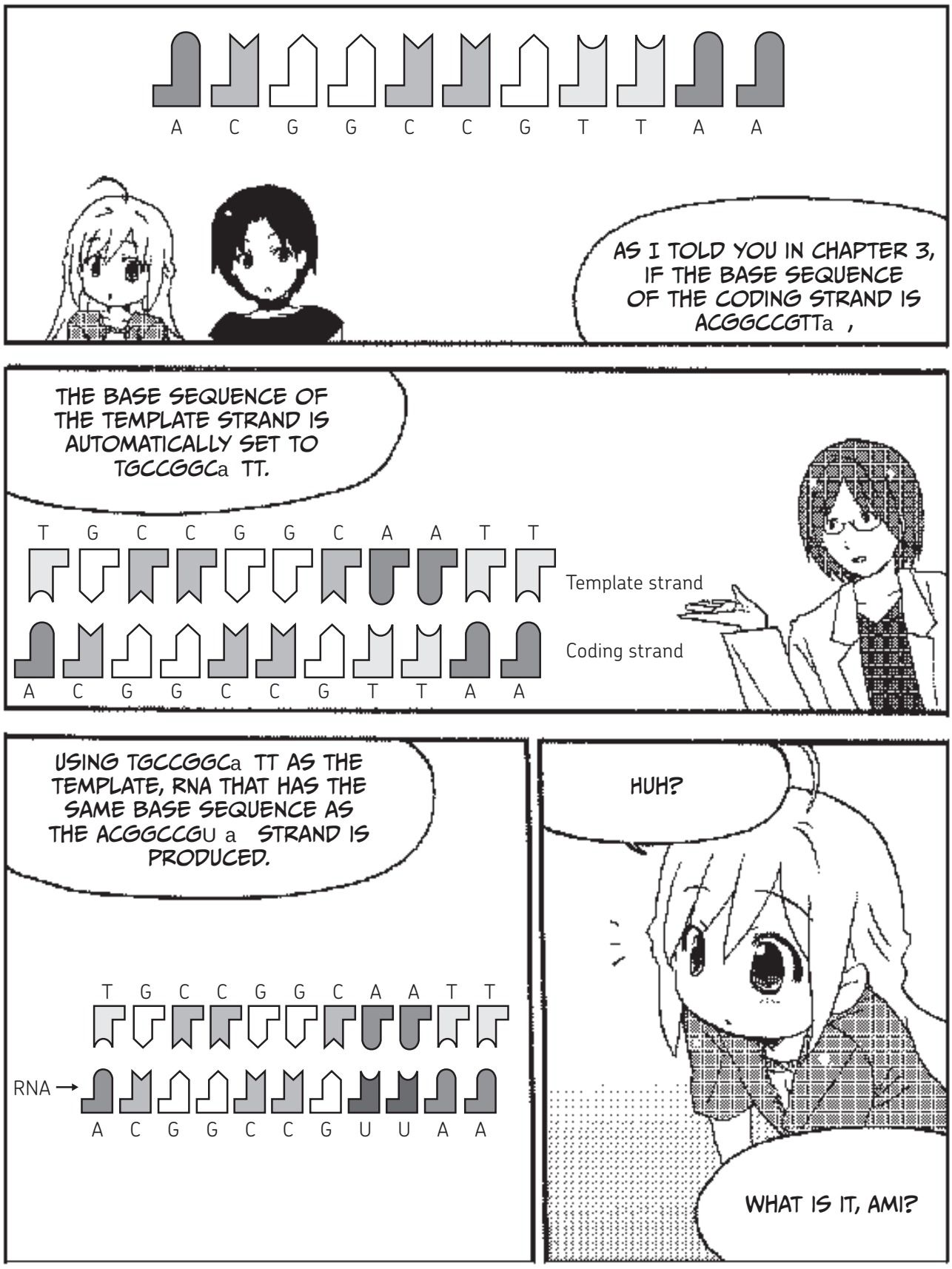

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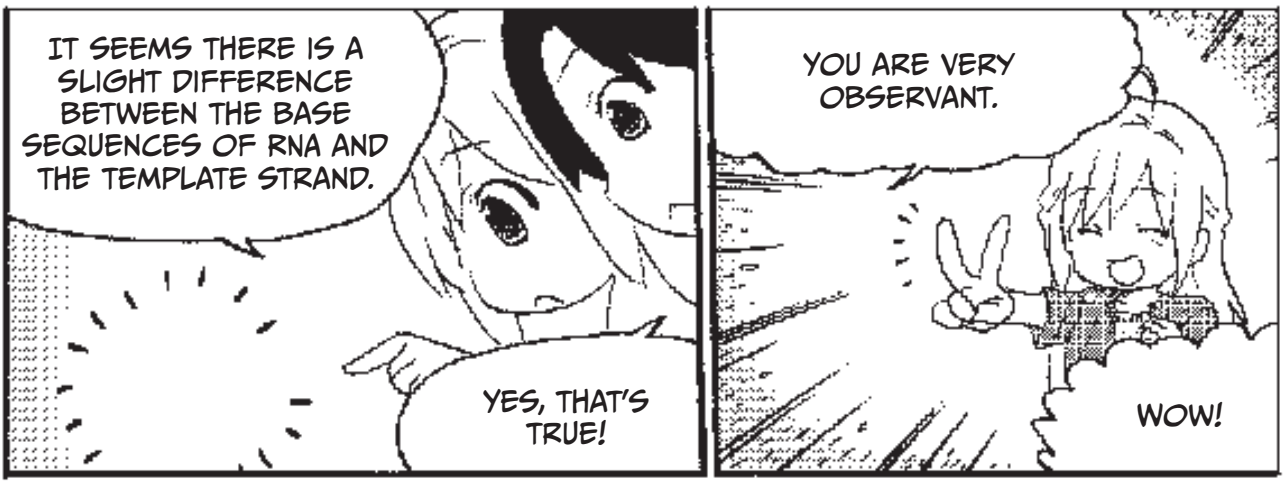
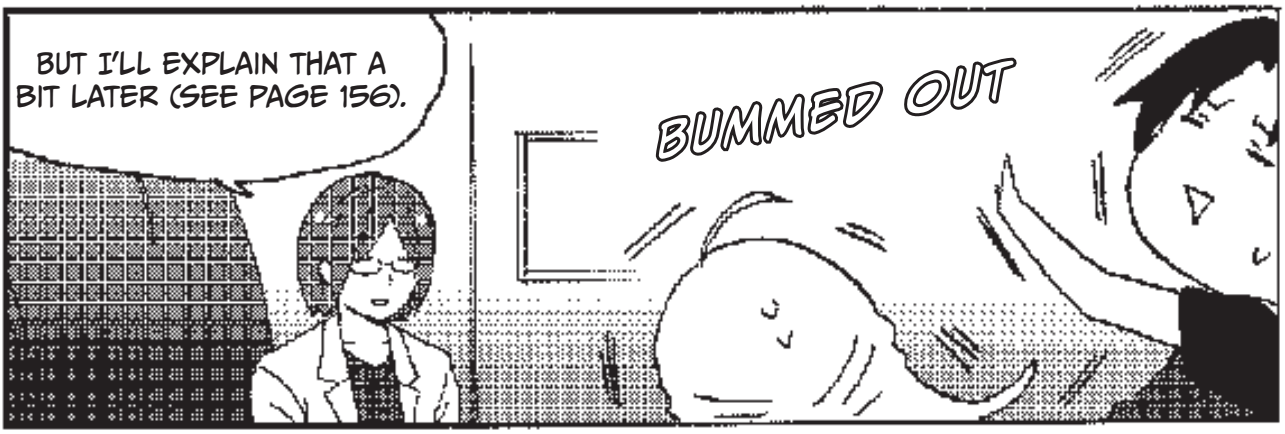
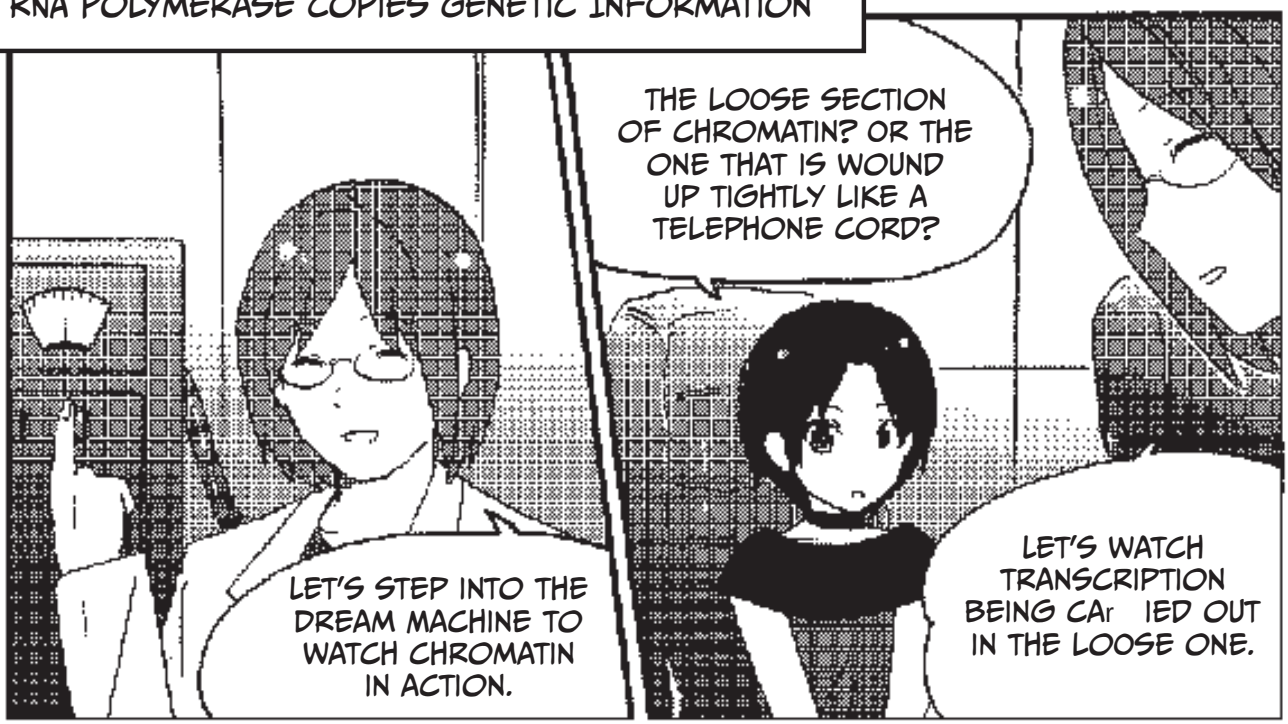




RNA POLYMERASE COPIES GENETIC INFORMATION



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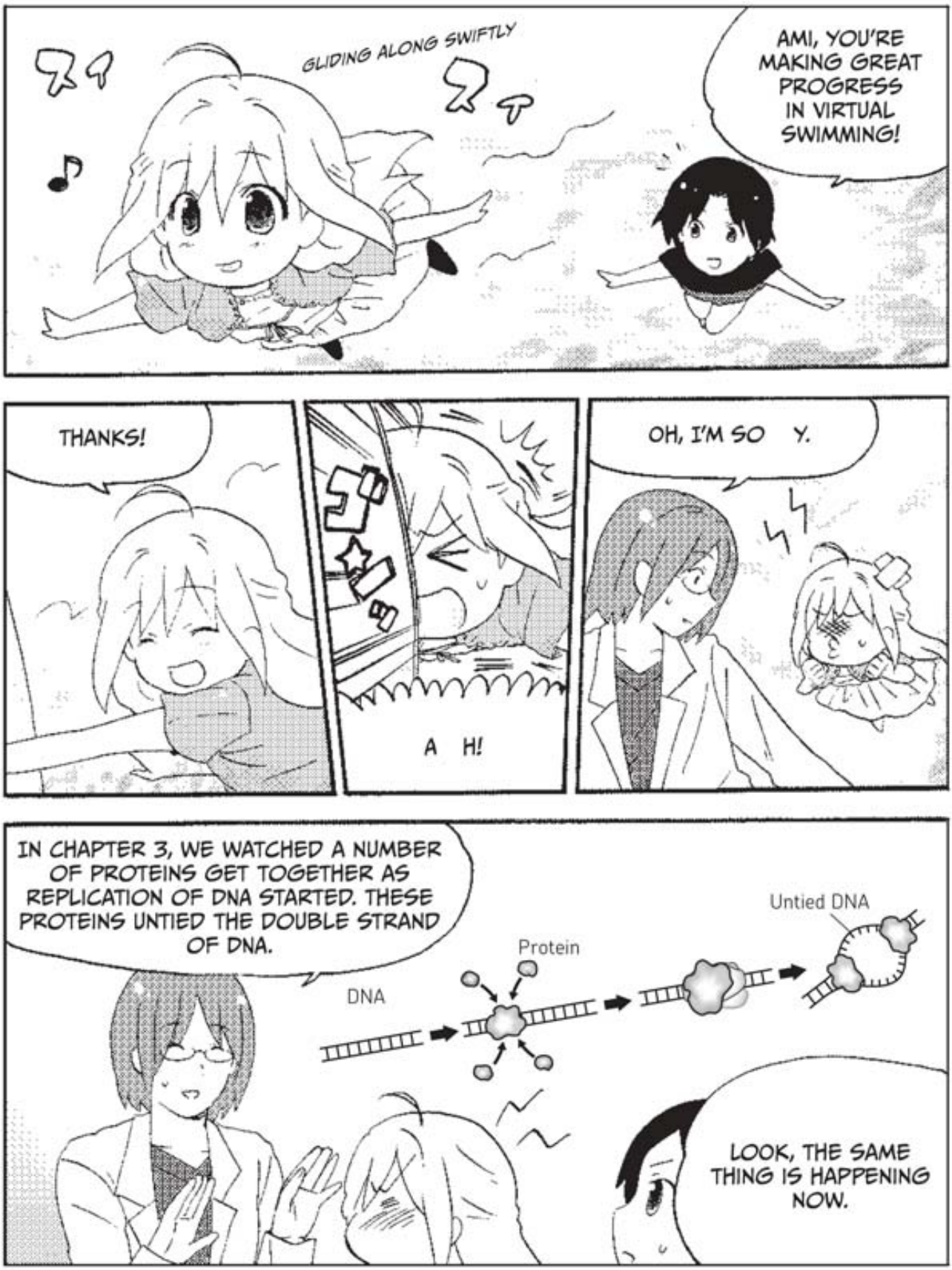

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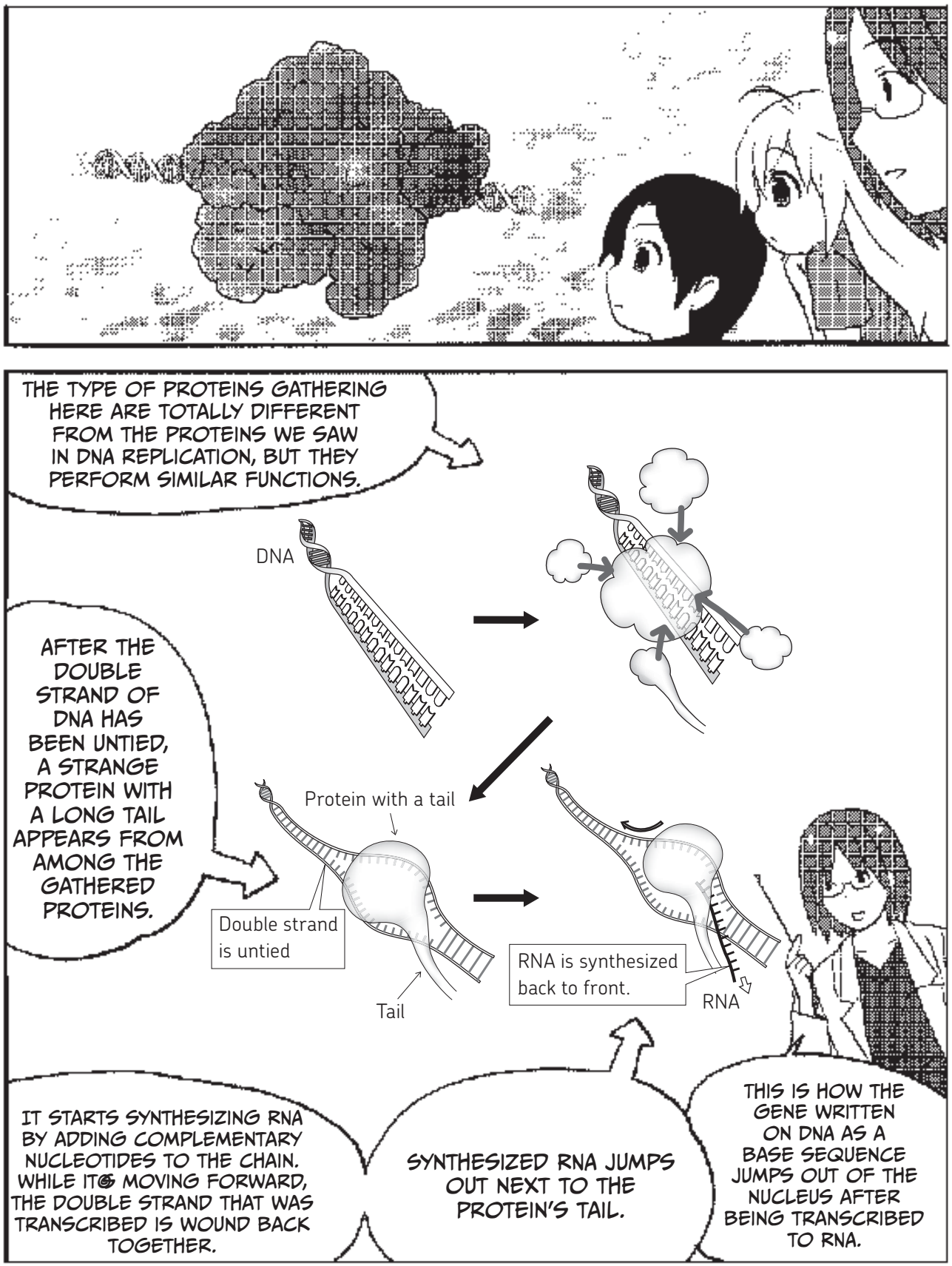

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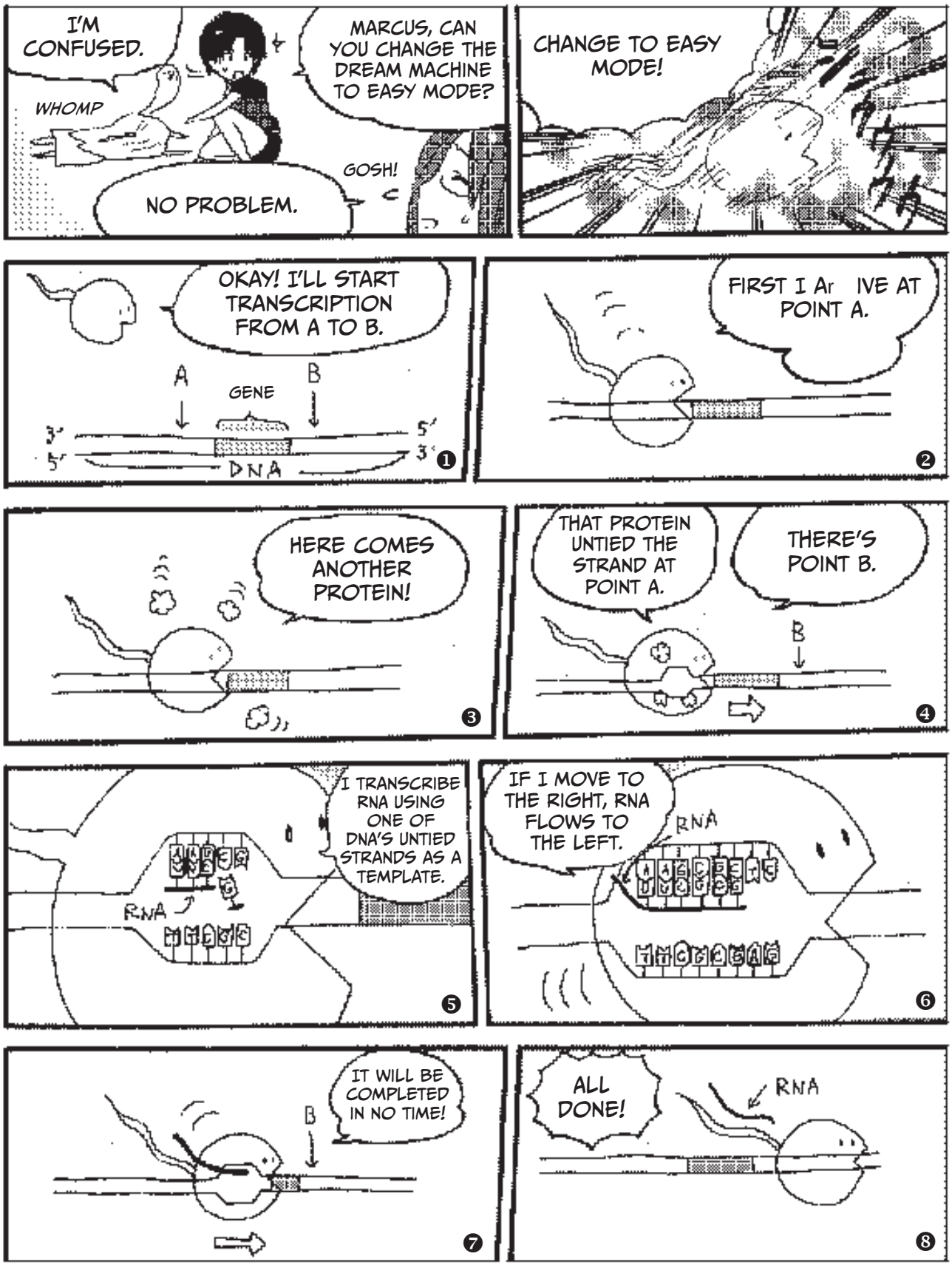

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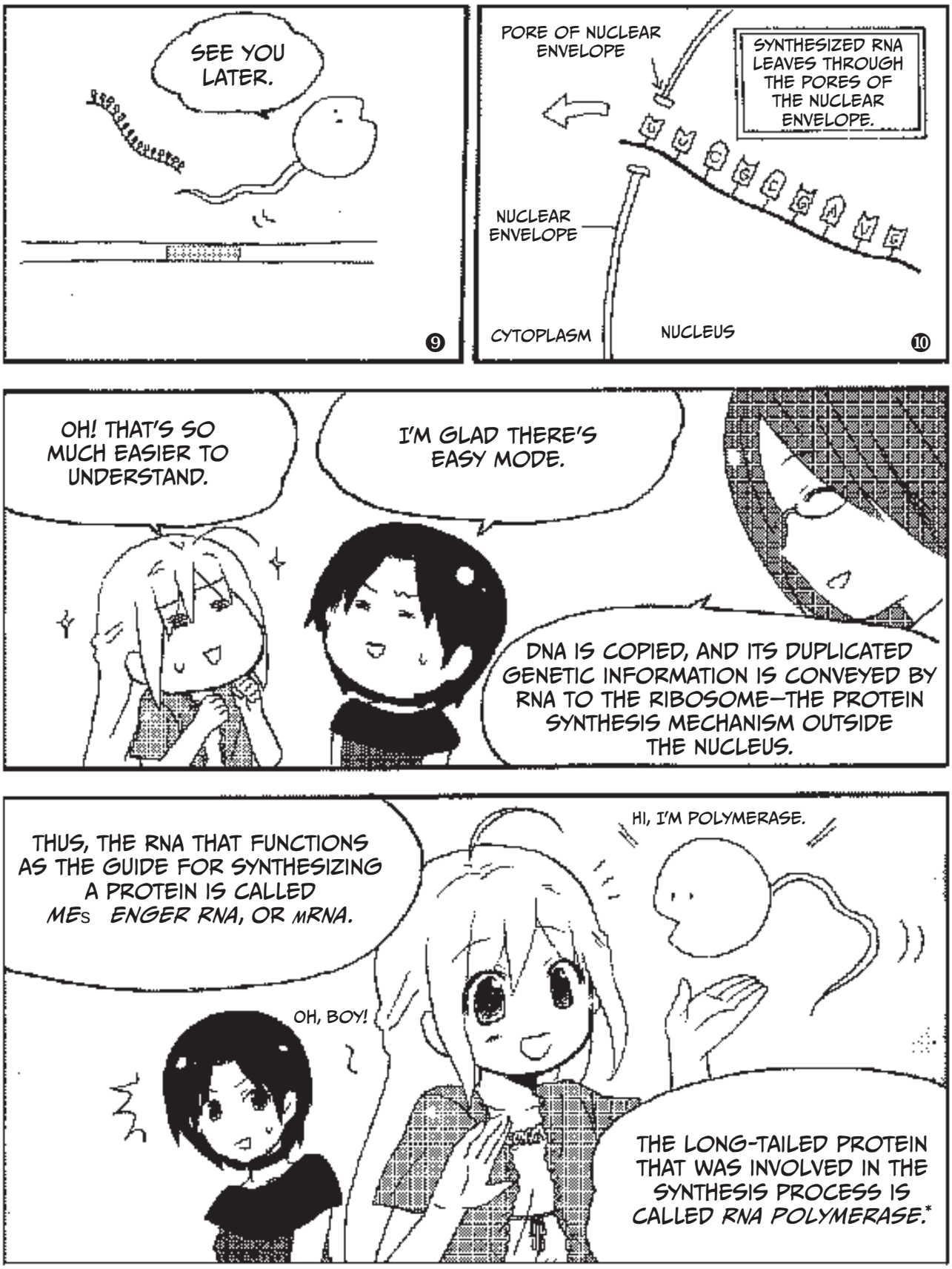

* TO BE EXACT, THE PROTEIN IN EUKARYOTIC ORGANISMS IS CALLED *RNA POLYMERASE II*.

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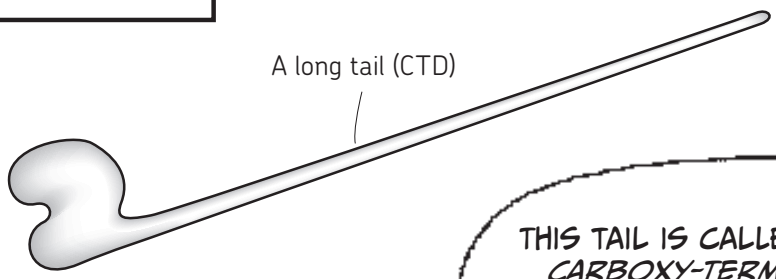
TRIMMING THE TRANSCRIBED mRNA

RNA POLYMERASE HAS A LONG TAIL.



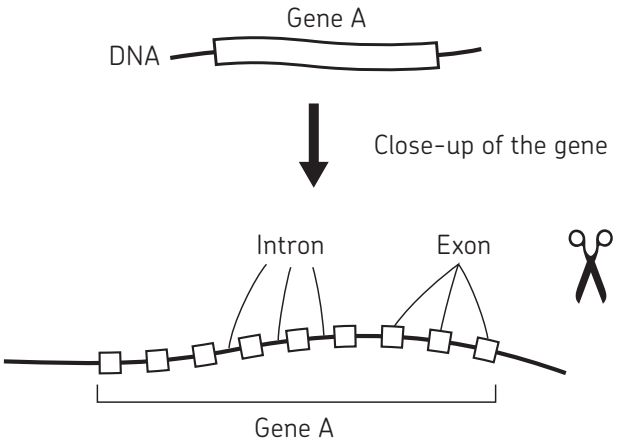
THIS TAIL IS CALLED THE *CARBOXY-TERMINAL DOMAIN*, OR *CTD*.

SYNTHESIZED RNA JUMPS OUT ALONG THE TAIL OF RNA POLYMERASE.

A LOT HAPPENS TO THE RNA AFTER THE POLYMERASE PLACES THE NUCLEOTIDES IN A SEQUENCE. LET'S TAKE A CLOSER LOOK.

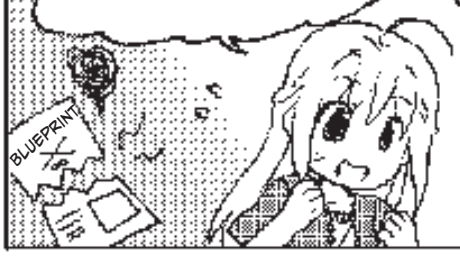
WELL, SEVERAL THINGS ARE HAPPENING. I'LL TALK ABOUT ONE OF THEM.

GENES, OR THE BLUEPRINTS OF PROTEIN WRITTEN ON DNA, ARE CUT INTO CHUNKS.



CUT?

IS THE BLUEPRINT CUT UP?



WHOA

YES. GENES ARE MADE UP OF INTRONS AND EXONS. THE INTRONS ARE NONCODING REGIONS OF NUCLEIC ACIDS AND ARE DELETED FROM THE RNA SEQUENCE. THE REMAINING SEQUENCE IS NOW THE MESSENGER RNA THAT CODES FOR A PROTEIN.

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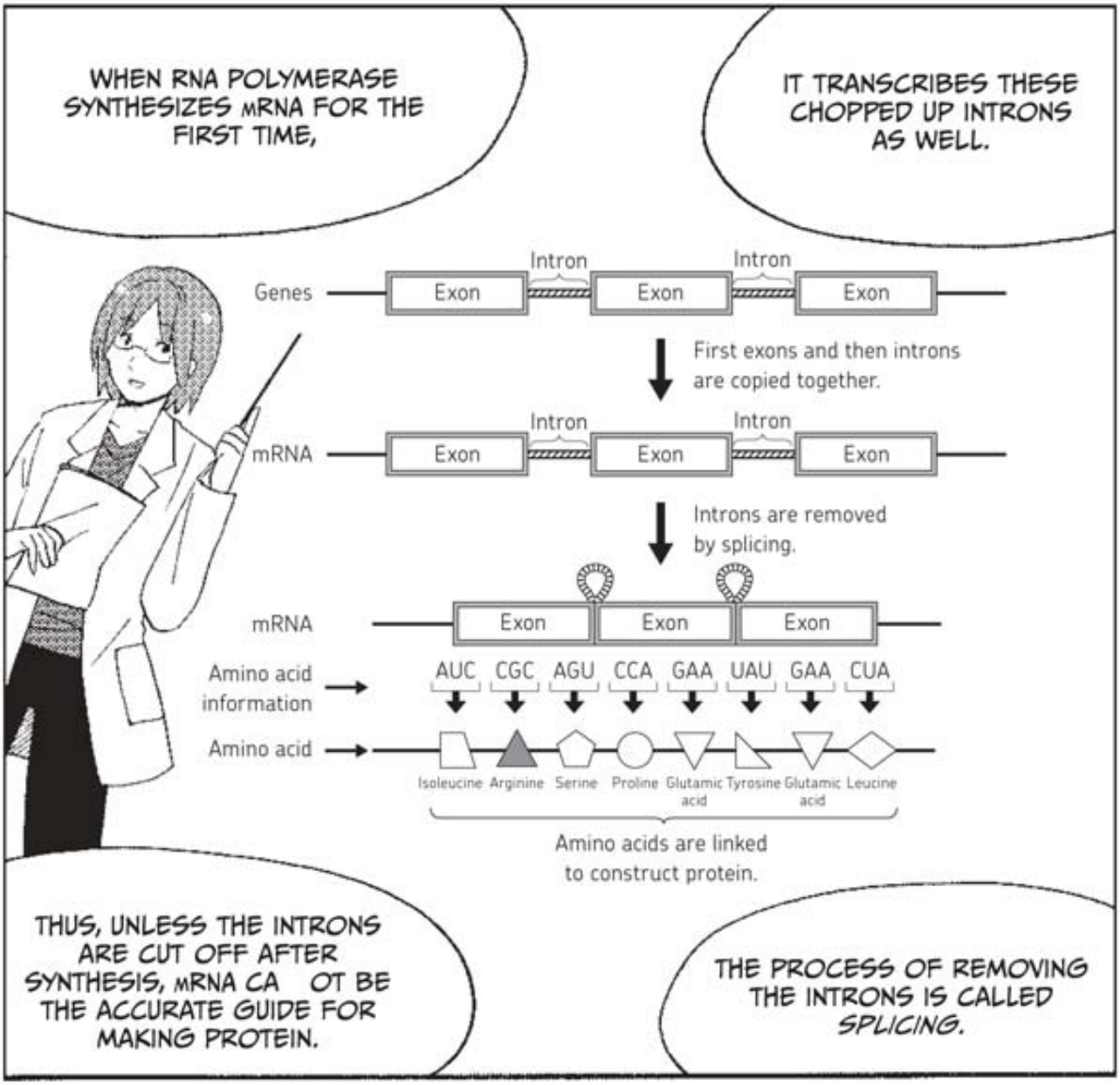

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EXON SHUFFLING

Marcus, why is a gene cut up?

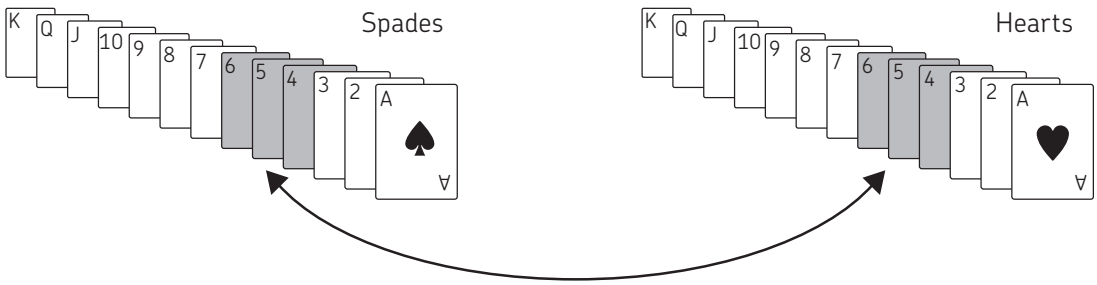
This is still being studied, and various theories have been presented.

Primitive organisms like bacteria do not have introns. Spliced genes allow easier mixing, which probably facilitated evolution.

Are genes mixed for evolution?

Imagine that a gene consists of a set of playing cards. If you can imagine that the cards from the ace to the king of spades make up a gene, and the cards from the ace to the king of hearts make up another gene, and that each card corresponds to an exon, the genes of hearts and spades consist of 13 exons each. (The space between each card is filled with introns.)

During the evolutionary process, the 4, 5, and 6 of spades could be replaced with the 4, 5, and 6 of hearts.



Each exon is made up of several base pairs. The introns between the cards allow the exons to switch without interrupting the sequence of any individual exon. This is what creates diversity among members of the same species.

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When the replacement of exons occurs between two genes, a new gene is produced. In human genes, very similar exons exist in two totally irrelevant genes. This indicates that genes each with different functions have been produced through the mixing of exons.

This process, called *exon shuffling*, is thought to have played a part in the evolution of living organisms through the diversification of genes.

Uuuuuhhhhh...

Sorry if that was a little difficult to understand.

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WHAT IS RNA?

CHARACTERS OF RNA

Do you understand that RNA is produced through DNA transcription?

Yes. A copy of DNA is RNA, right? Does that mean DNA and RNA are totally identical?

No, they're not. Ami, you noticed a little while ago that RNA is slightly different from the template strand of DNA.

Right!

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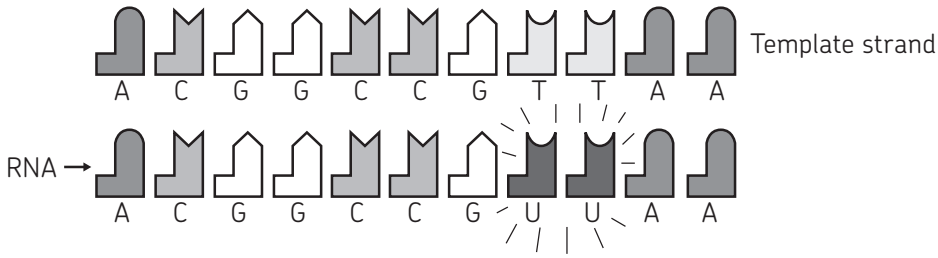


I'll explain the difference between RNA and DNA.

In Chapter 2, you learned about the four types of characters for DNA: adenine (A), guanine (G), cytosine (C), and thymine (T).

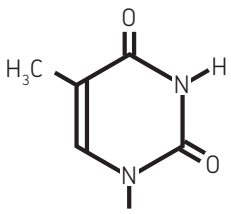
And you also learned that a gene is a code represented by a sequence made up of these four types of characters (the base sequence).

Does that mean the same code is written on RNA?

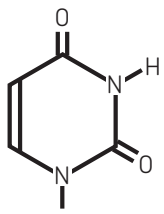
The answer is both "Yes" and "No."

What?!

There are four types of characters (bases) for RNA; three of them—A, G, and C—are the same as that of DNA. The fourth character is different; it's not T (thymine) as you would expect, but U (uracil).



Thymine (T)



Uracil (U)

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Why is that one different?

This is currently being studied, but the following hypothesis is supported by many researchers.

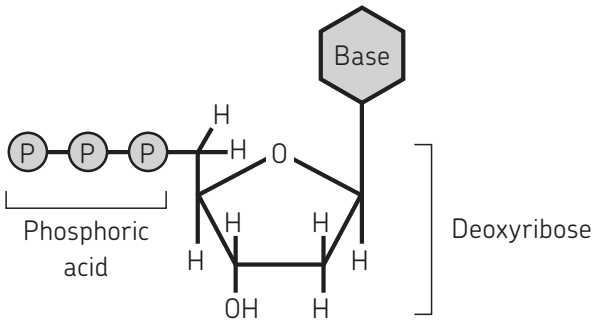
During DNA replication, DNA polymerase checks for mutations in the DNA. U (uracil) can be produced through the mutation of C (cytosine). When DNA encounters a uracil during this check for mutations, it would be "confused." DNA polymerase would have no way of knowing if it were a mutated cytosine that needs to be corrected, or if it is really supposed to be uracil. It's possible that the wrong kind of repair could be conducted.

That's why DNA has developed T (thymine), which is easier to identify.

Yes, but this is still a hypothesis. There are many more things waiting to be discovered in the world of molecular biology.

DNA AND RNA USE DIFFERENT SUGARS

In Chapter 2, you learned that a nucleotide (deoxyribonucleotide), the material that makes up DNA, consists of phosphoric acid, deoxyribose (a type of sugar), and a base.



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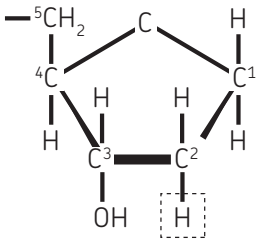
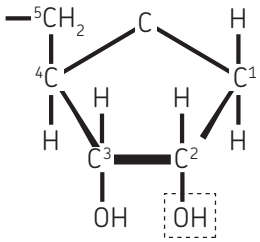
RNA is made up of a special kind of nucleotide, called a *ribonucleotide*. It is also made up of phosphoric acid, sugar, and a base. This sugar is not deoxyribose but simply *ribose*.



Deoxyribose
(Material of DNA)



Ribose
(Material of RNA)

In addition to the difference in one of the characters, thymine or uracil, there is another variation between DNA and RNA. DNA uses deoxyribose as a sugar, and RNA uses ribose.

What's the difference between deoxyribose and ribose?

They differ only in one point, whether hydrogen (H) or hydroxyl (OH) becomes the second carbon (C). Hydrogen binds to the second carbon with deoxyribose, and hydroxyl binds to the second carbon with ribose. This is the only difference, but it causes big changes in the properties of DNA and RNA in molecular form.

How do their properties differ?

The reactivity of RNA containing hydroxyl is much higher than that of DNA. This is because the oxygen atom (O) in hydroxyl is more reactive with other atoms.

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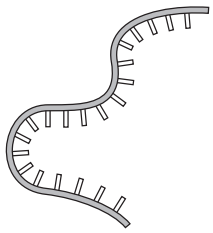
RNA IS FLEXIBLE

As described earlier, we can list two differences that cause chemical differences between RNA molecules and DNA molecules. They are the differences in the base, namely T (thymine) or U (uracil), and the differences in the sugar of the nucleotide. There is another point that contributes significantly to the differences between them. I've explained that DNA forms double strands, but most RNA moves around as a single strand.

DNA has a double strand.



RNA has a single strand.

Really? Why? Double strands are more useful, aren't they?

This is definitely the case with DNA. But it is advantageous for RNA to be a single strand. A single strand allows RNA to be flexible without being bound by the fixed structure of the double helix. RNA can thus transform into various shapes within a single molecule.

Suppose there is a base sequence AGGCCC and another base sequence GGGCCU somewhere on a single RNA.

Since A and U, and G and C can find and create pairs together, this portion alone in the molecule has a double-strand structure. Then, RNA will be shaped like this.

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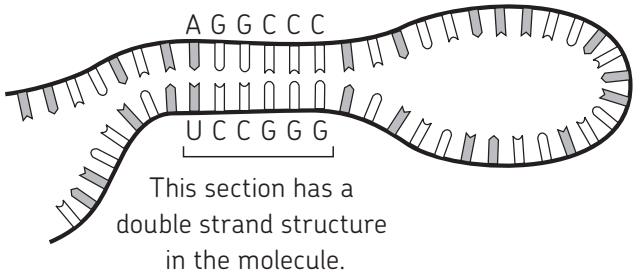
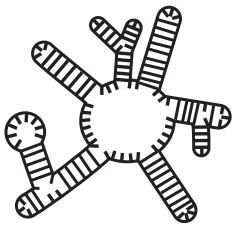
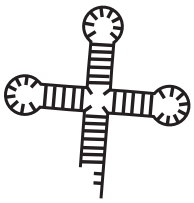
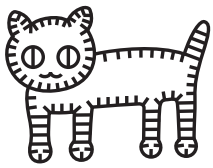


This section has a double strand structure in the molecule.

RNA is not just a string-like molecule; it can transform into various shapes just by changing the base sequence. Since RNA can take various forms, it is capable of assuming various roles in addition to merely functioning as a duplicate molecule. This is very important.







THERE ARE MANY TYPES OF RNA

Various roles—like what?

Messenger RNA (mRNA) is not the only kind of RNA you will find. RNA can take the form of *transfer RNA (tRNA)* and *ribosomal RNA (rRNA)* as well. Both play a very important role when constructing protein from the genetic information copied to mRNA.

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It sounds very mysterious. I have heard the word DNA but I didn't know anything about RNA. Actually, RNA looks like it does a whole lot more than DNA.

Yes, you're right. Since RNA is more flexible...

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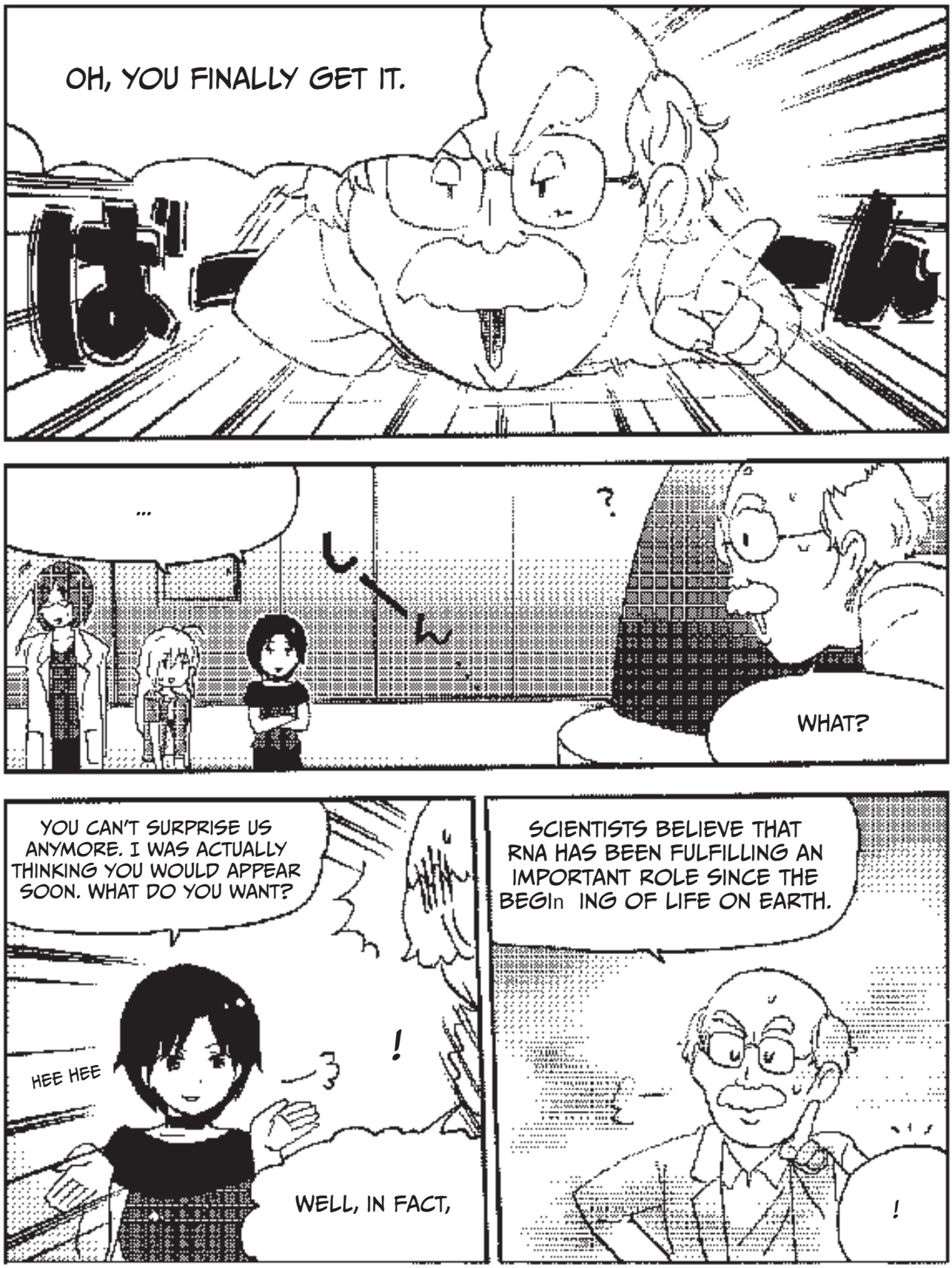

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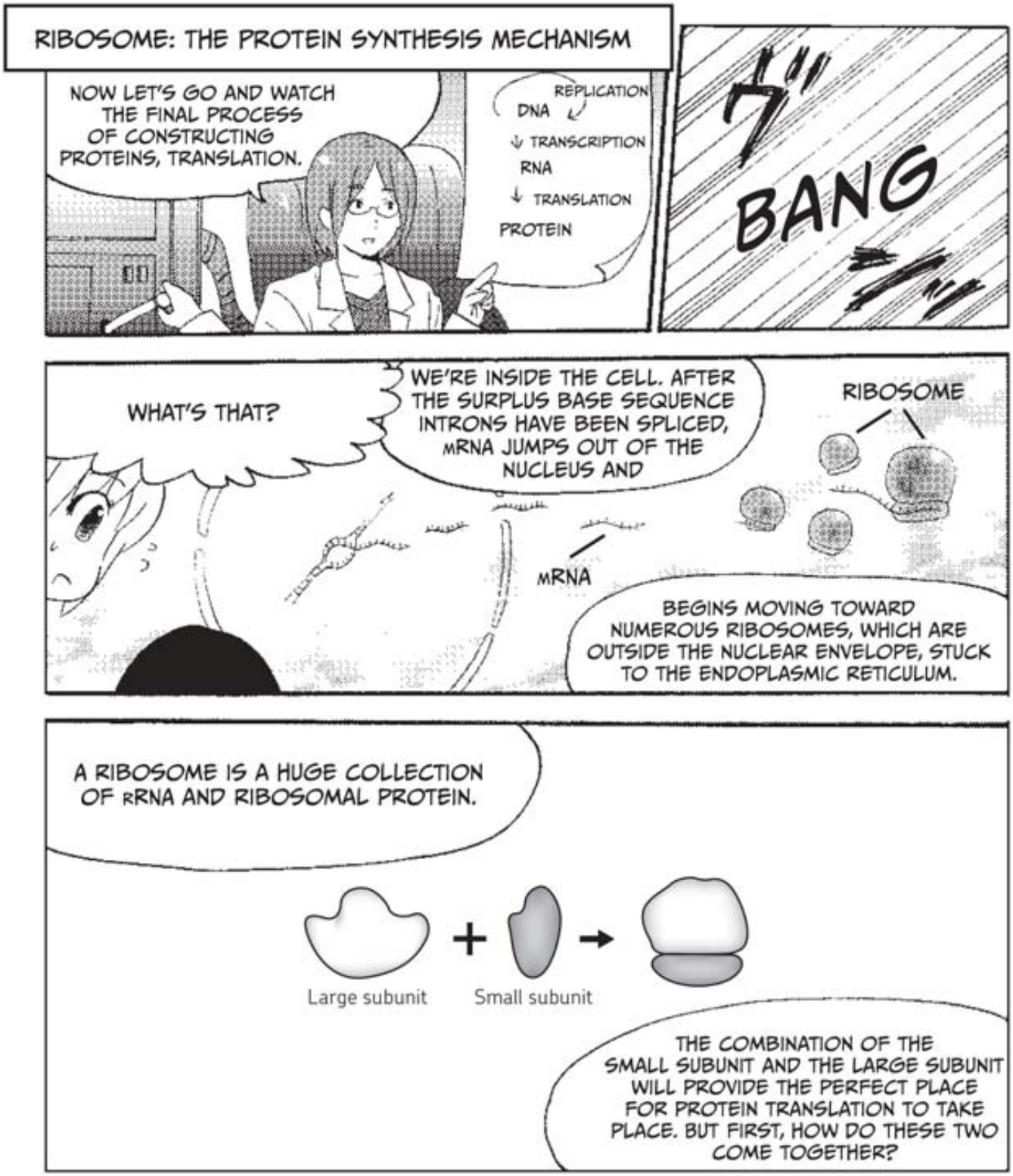
TRANSFER RNA



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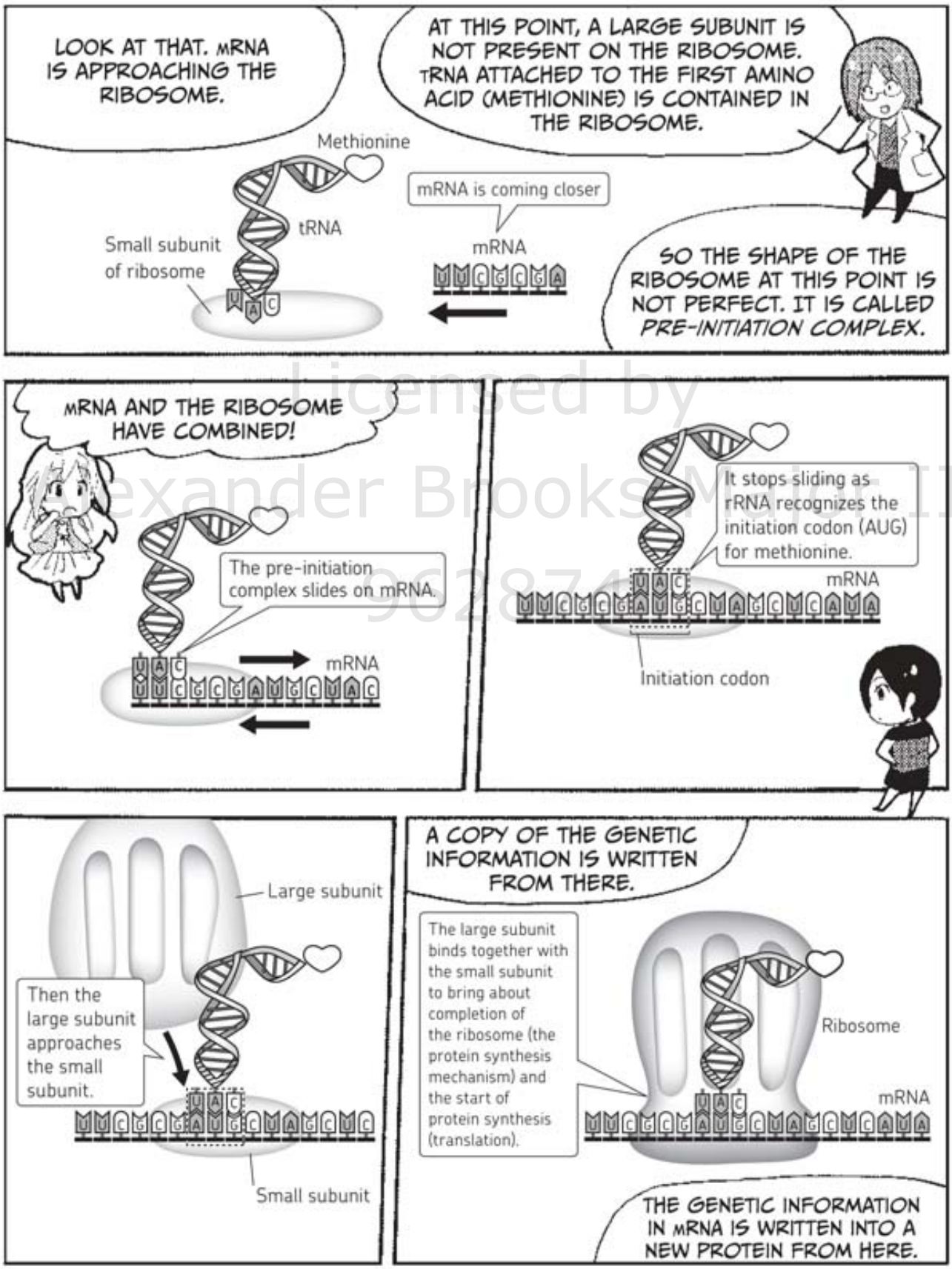

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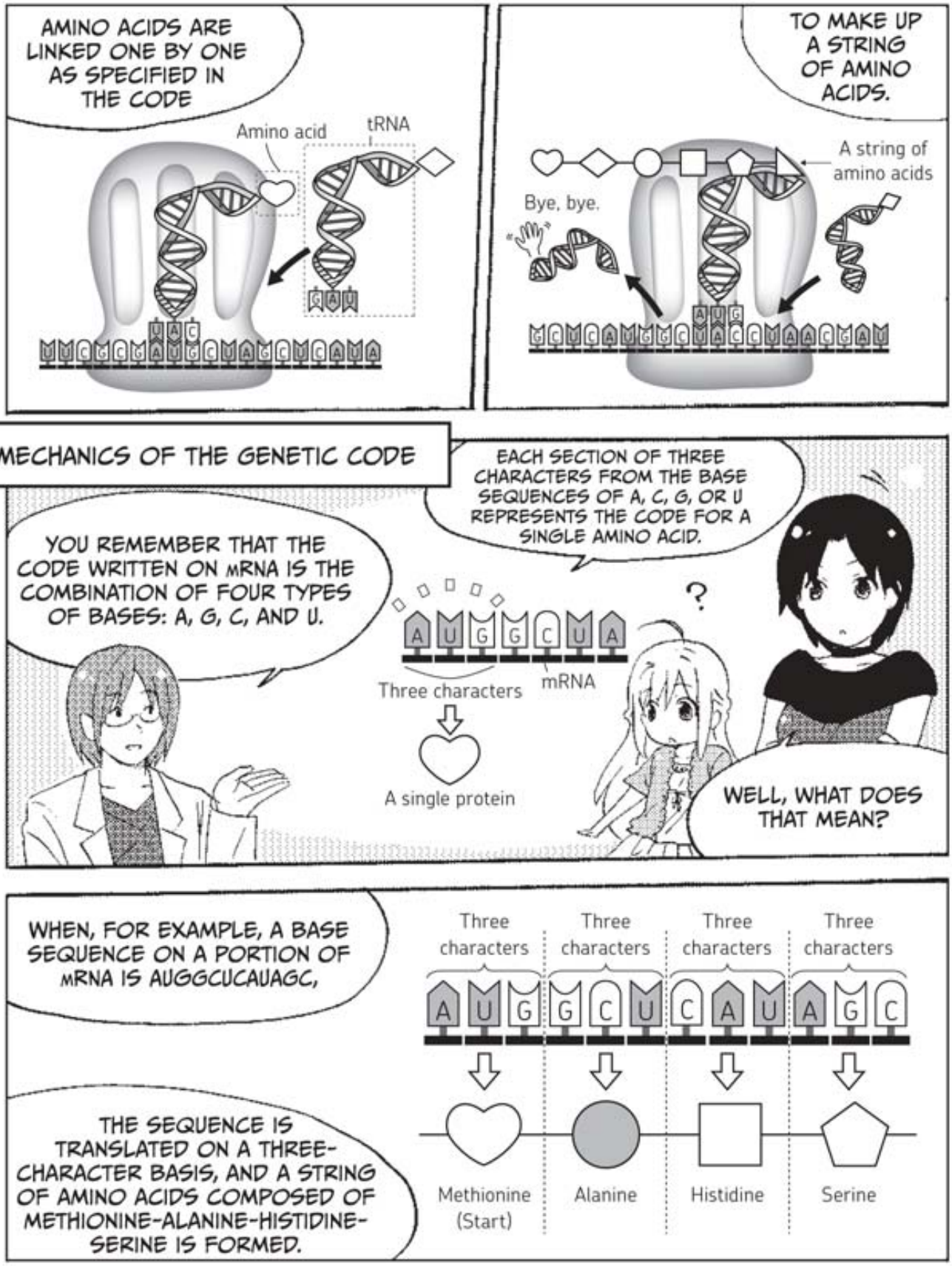

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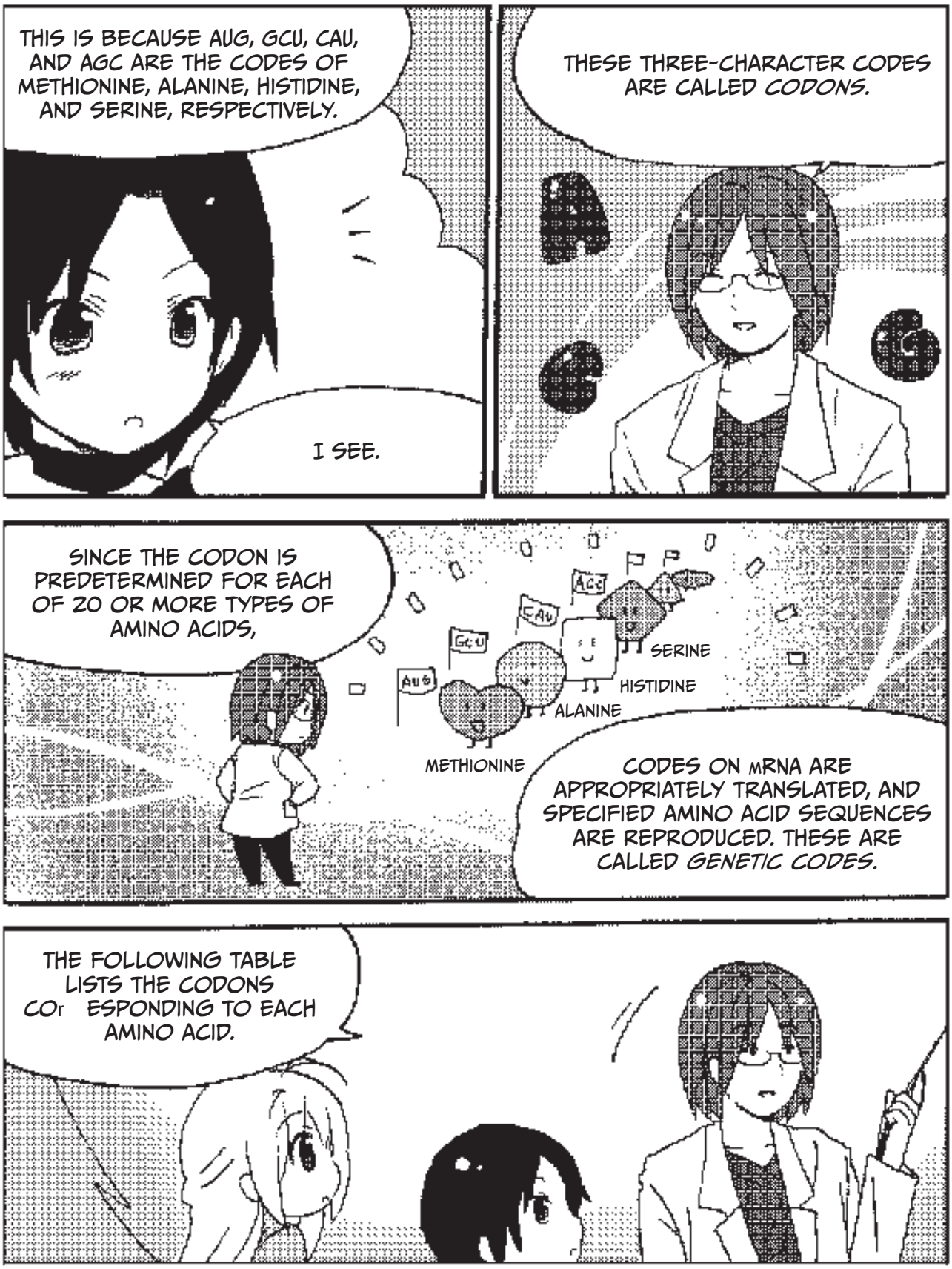

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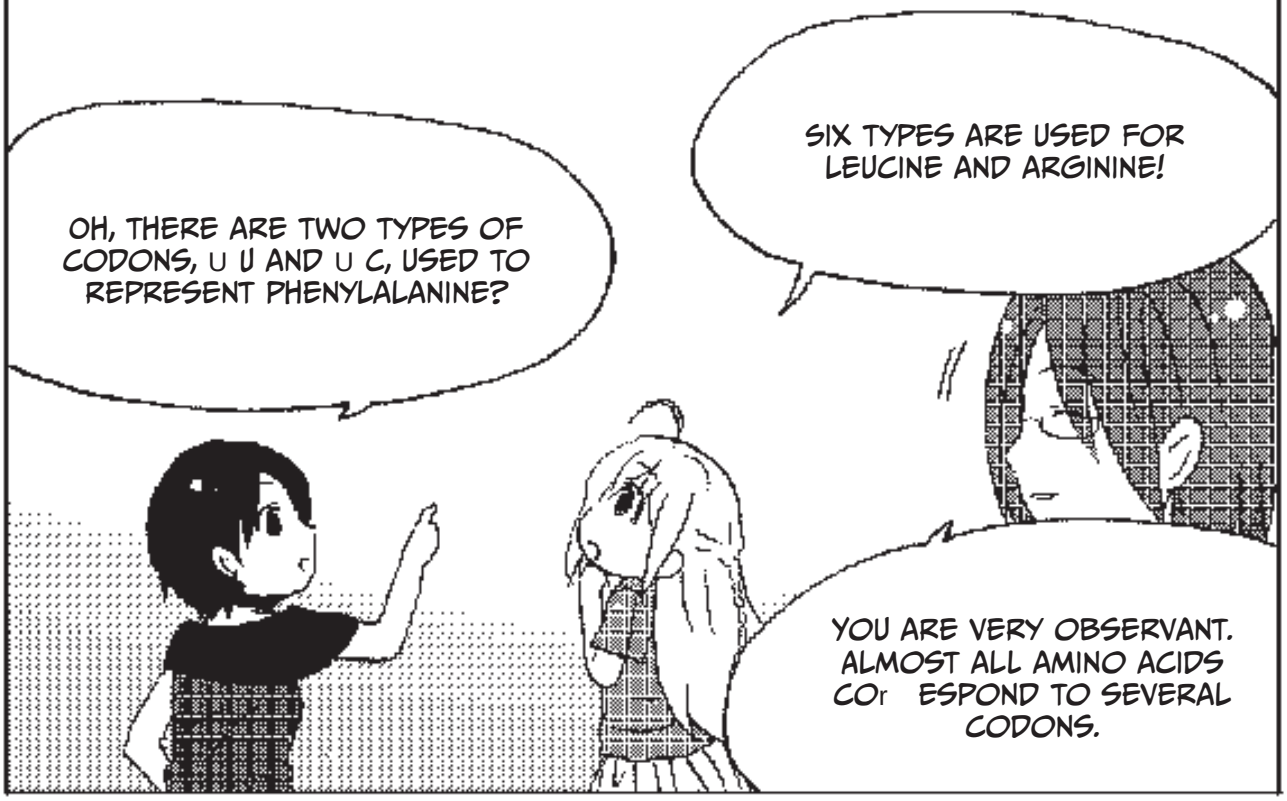
First character	Second character U	Second character C	Second character A	Second character G	Third character
U	(UUU) Phenylalanine	(UCU) Serine	(UAU) Tyrosine	(UGU) Cysteine	U
	(UUC) Phenylalanine	(UCC) Serine	(UAC) Tyrosine	(UGC) Cysteine	C
	(UUA) Leucine	(UCA) Serine	(UAA) Stop codon	(UGA) Stop codon	A
	(UUG) Leucine	(UCG) Serine	(UAG) Stop codon	(UGG) Tryptophane	G
C	(CUU) Leucine	(CCU) Proline	(CAU) Histidine	(CGU) Arginine	U
	(CUC) Leucine	(CCC) Proline	(CAC) Histidine	(CGC) Arginine	C
	(CUA) Leucine	(CCA) Proline	(CAA) Glutamine	(CGA) Arginine	A
	(CUG) Leucine	(CCG) Proline	(CAG) Glutamine	(CGG) Arginine	G
A	(AUU) Isoleucine	(ACU) Threonine	(AAU) Asparagine	(AGU) Serine	U
	(AUC) Isoleucine	(ACC) Threonine	(AAC) Asparagine	(AGC) Serine	C
	(AUA) Isoleucine	(ACA) Threonine	(AAA) Lycine	(AGA) Arginine	A
	(AUG) Methionine (start codon)	(ACG) Threonine	(AAG) Lycine	(AGG) Arginine	G
G	(GUU) Valine	(GCU) Alanine	(GAU) Asparagic acid	(GGU) Glycine	U
	(GUC) Valine	(GCC) Alanine	(GAC) Asparagic acid	(GGC) Glycine	C
	(GUA) Valine	(GCA) Alanine	(GAA) Glutamic acid	(GGA) Glycine	A
	(GUG) Valine	(GCG) Alanine	(GAG) Glutamic acid	(GGG) Glycine	G



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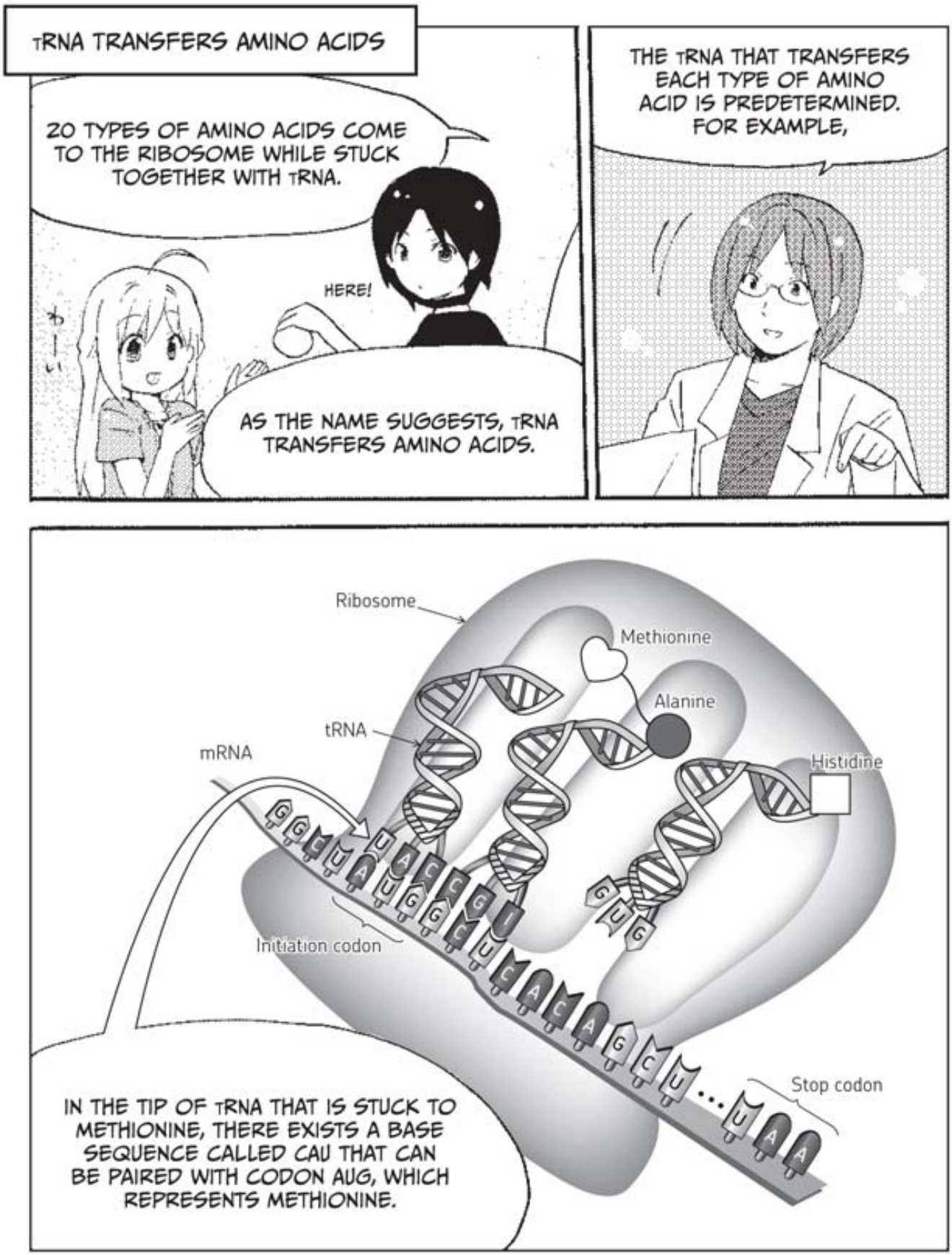

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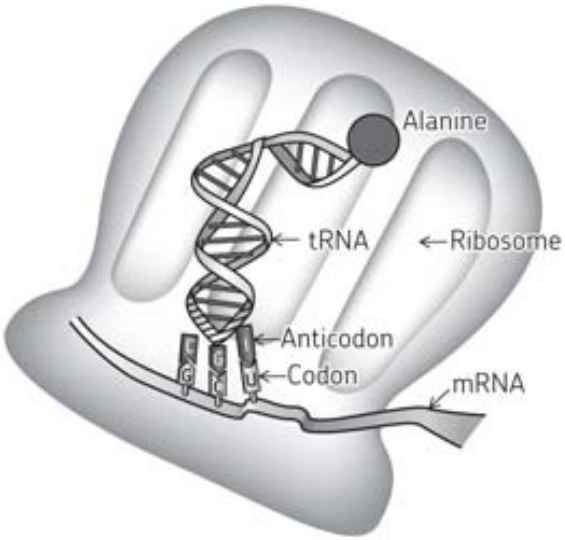
The three base sequences on tRNA that can be paired with this codon make up an *anticodon*.

Likewise, every tRNA that is bound to the amino acid alanine has a sequence (or anticodon) IGC that can be paired with GCU, the code for alanine from mRNA. For the first character of an anticodon, a strange character, *I* for inosine, is sometimes used.



Does "sometimes" mean it is a substitute for another base?

Well, inosine is a special base that can stick together with two or three types of bases. As I just described, the power of the third base of a codon for making a pair with the first base of an anticodon is weak. As a result, such a base can make a pair with other bases, too. Such base pairing is called *wobble base pairing*.

It's like the joker in a stack of playing cards!

Yes, I agree. You could say it's a wildcard.

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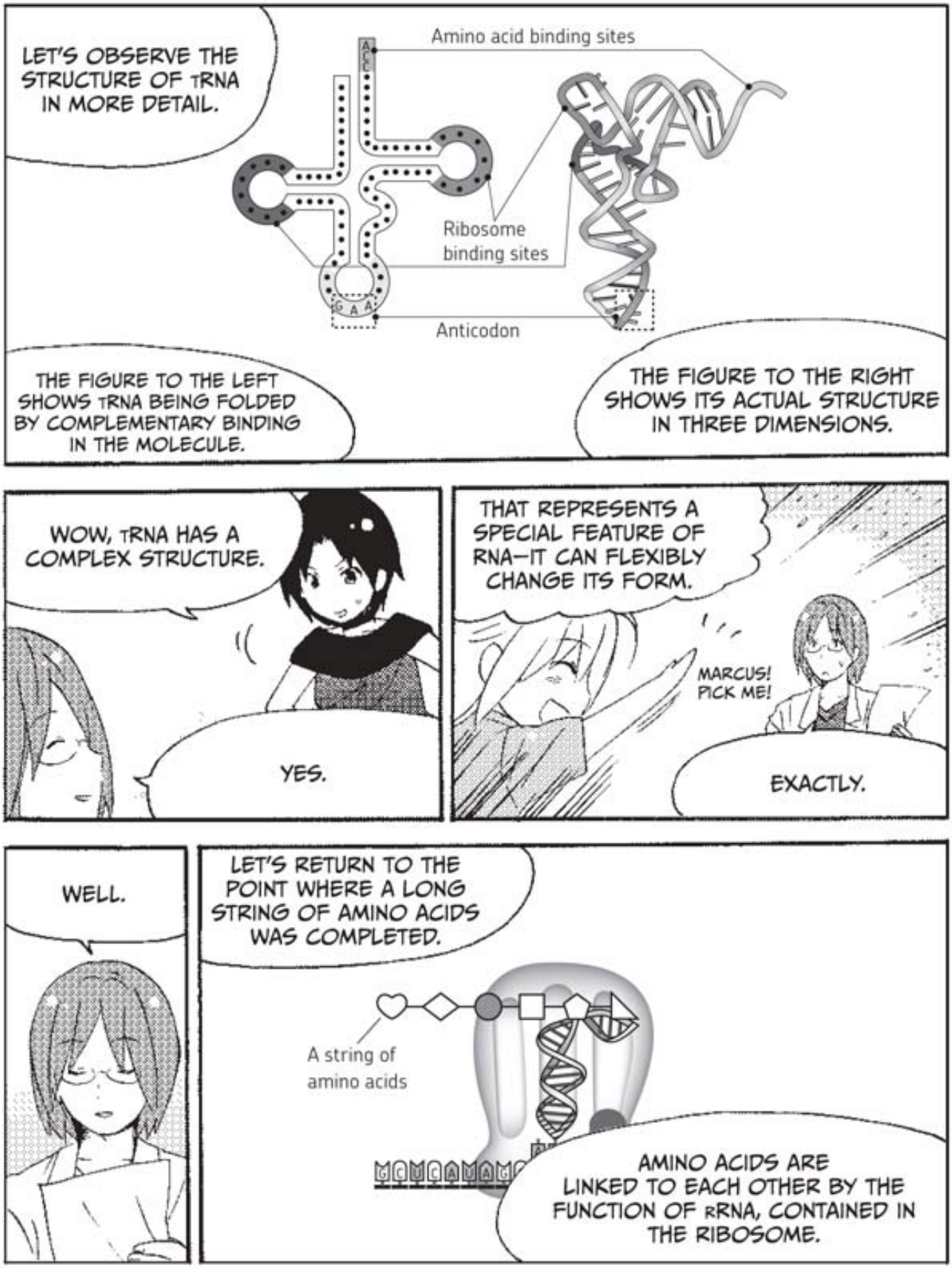

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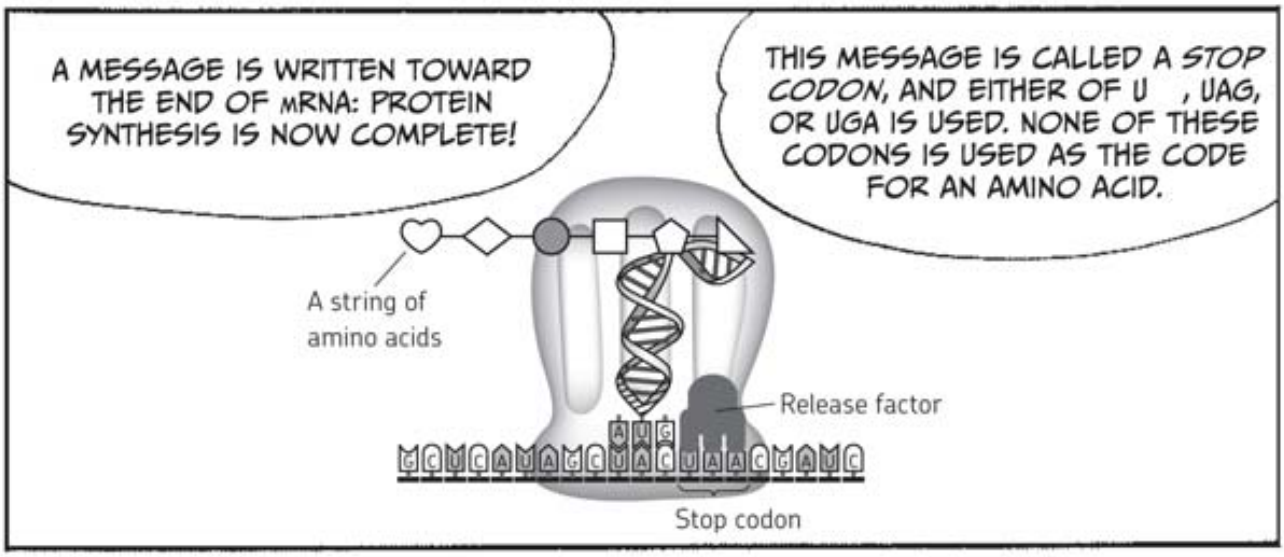

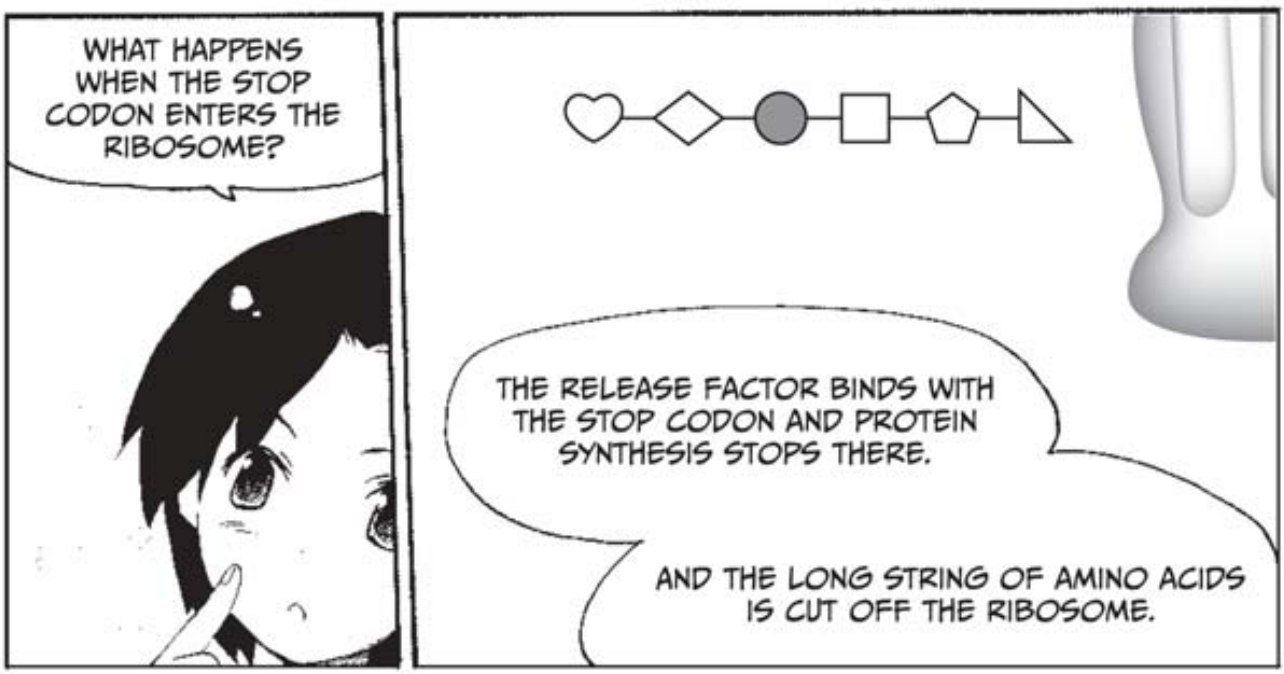

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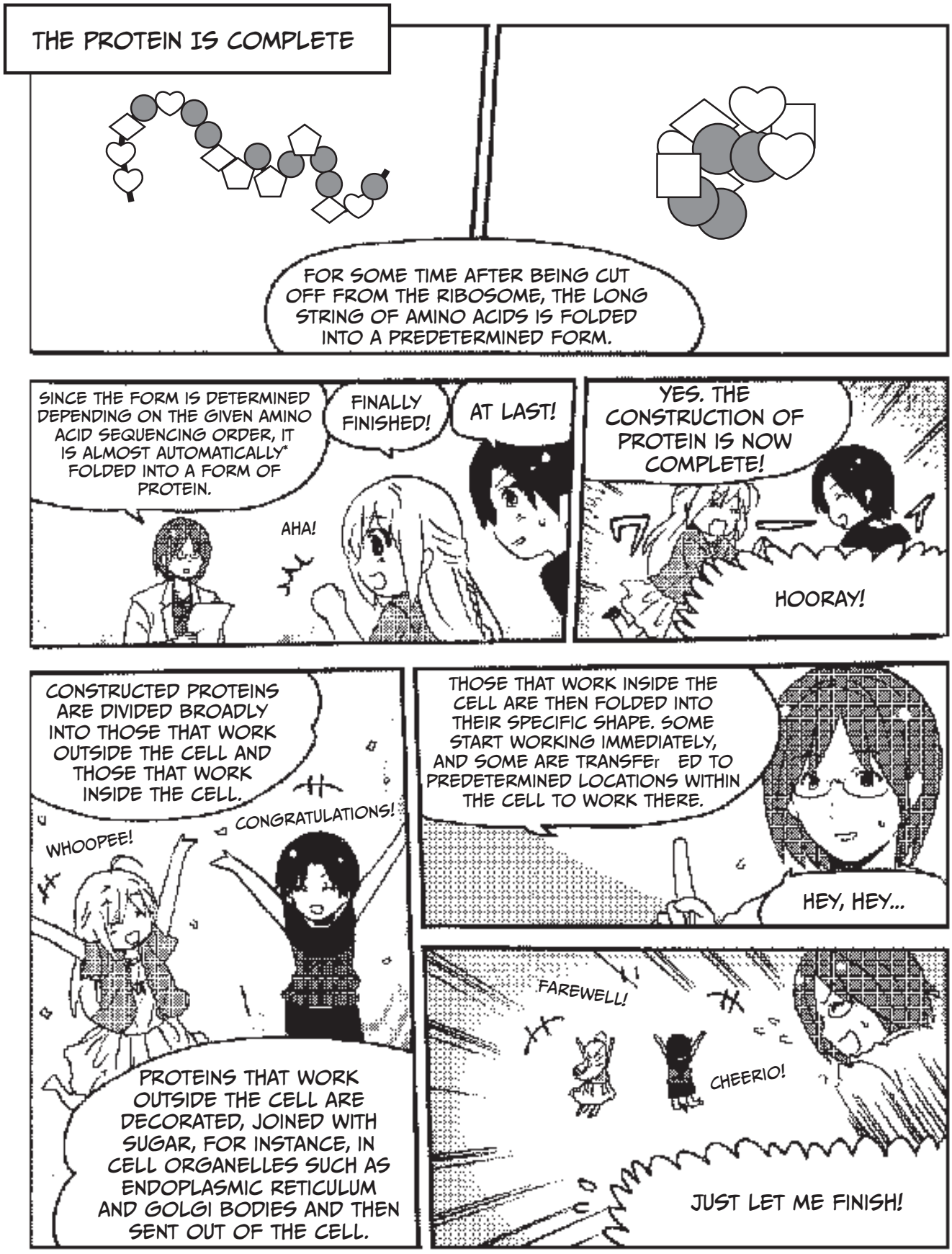

* TO BE EXACT, A PROTEIN IS NOT AUTOMATICALLY FOLDED. FOLDING IS CAr IED OUT WITH THE HELP OF OTHER PROTEINS.

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5

GENETIC TECHNOLOGY AND RESEARCH

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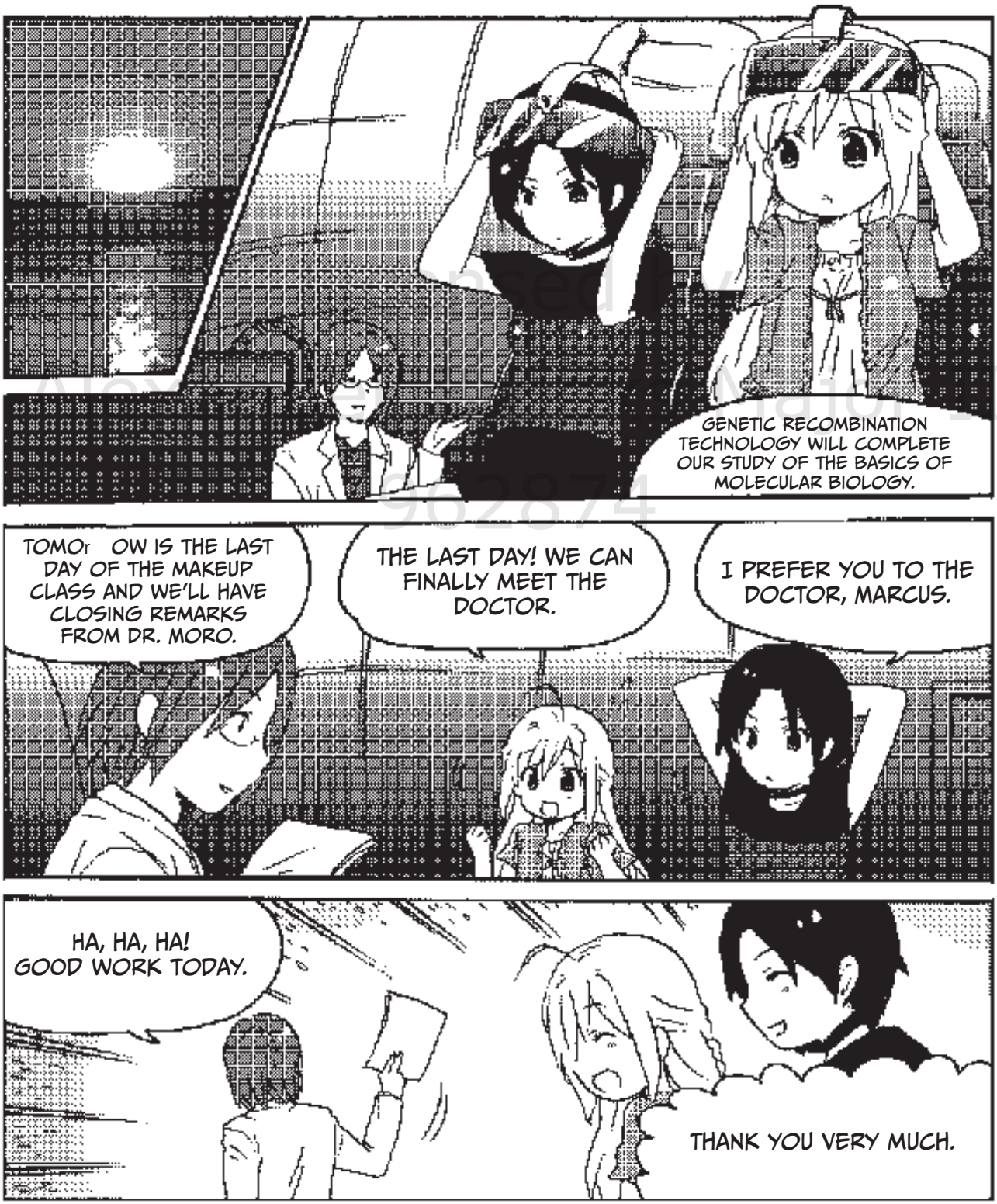
WHAT IS GENETIC RECOMBINATION TECHNOLOGY?



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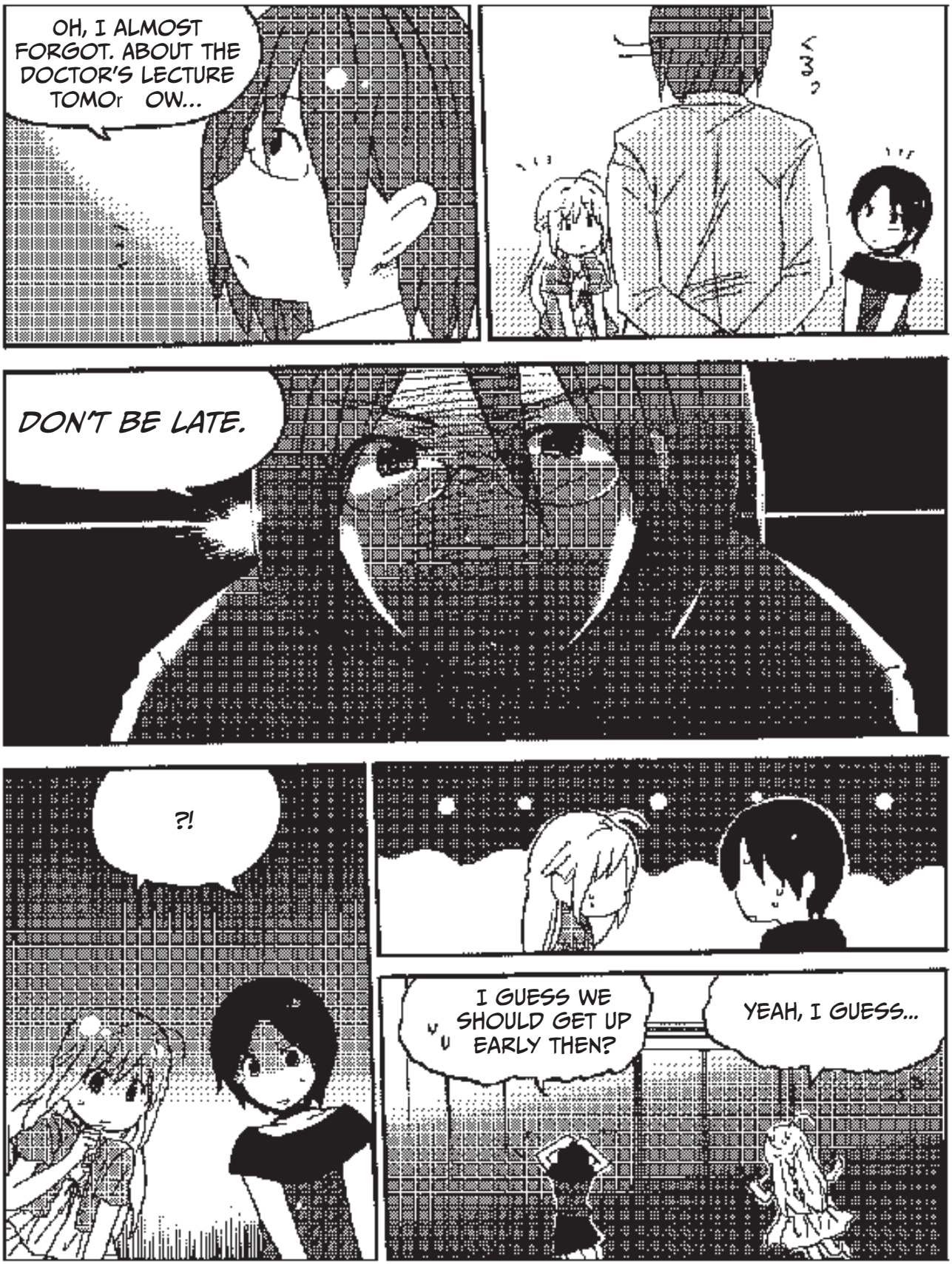

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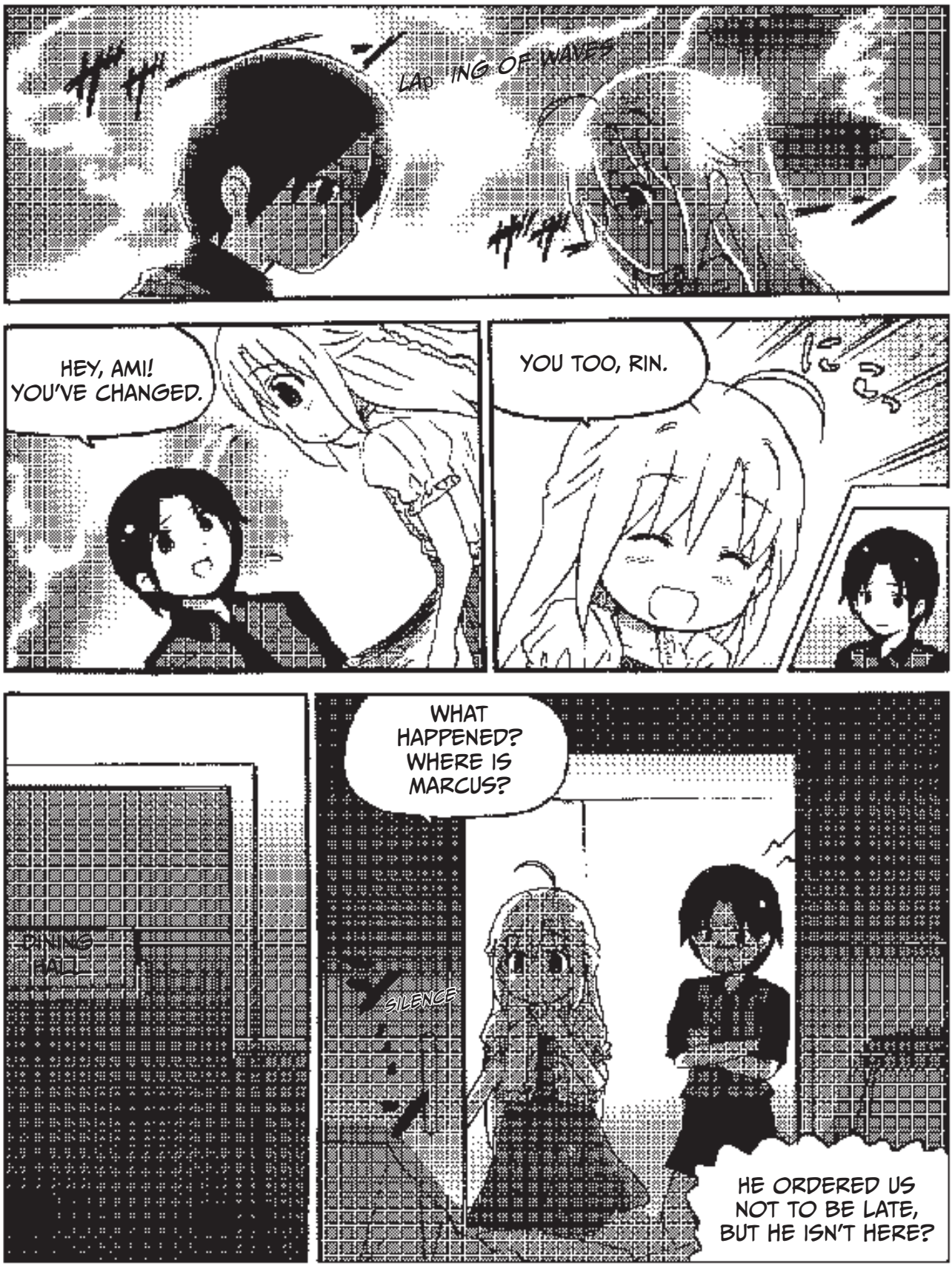

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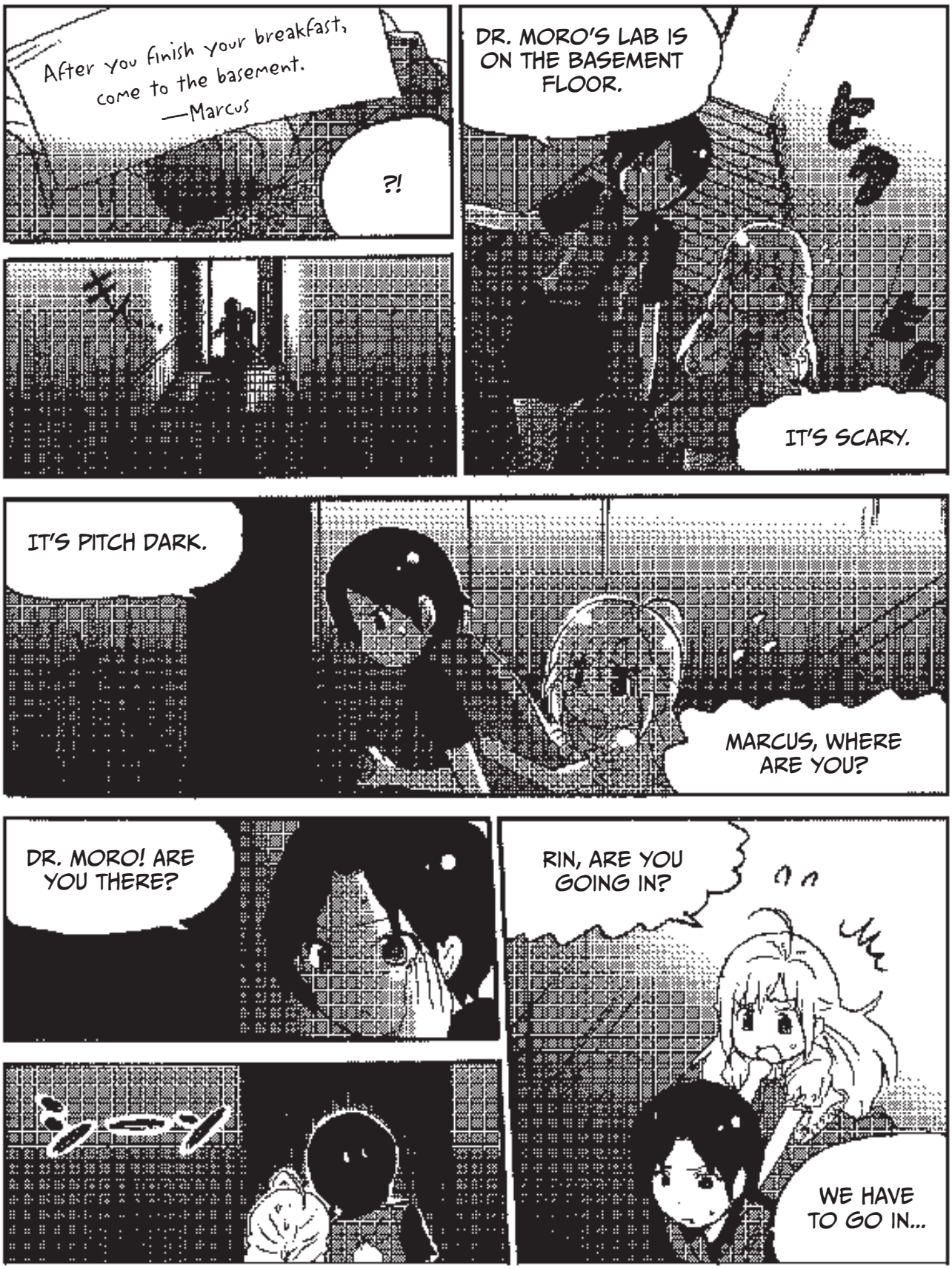

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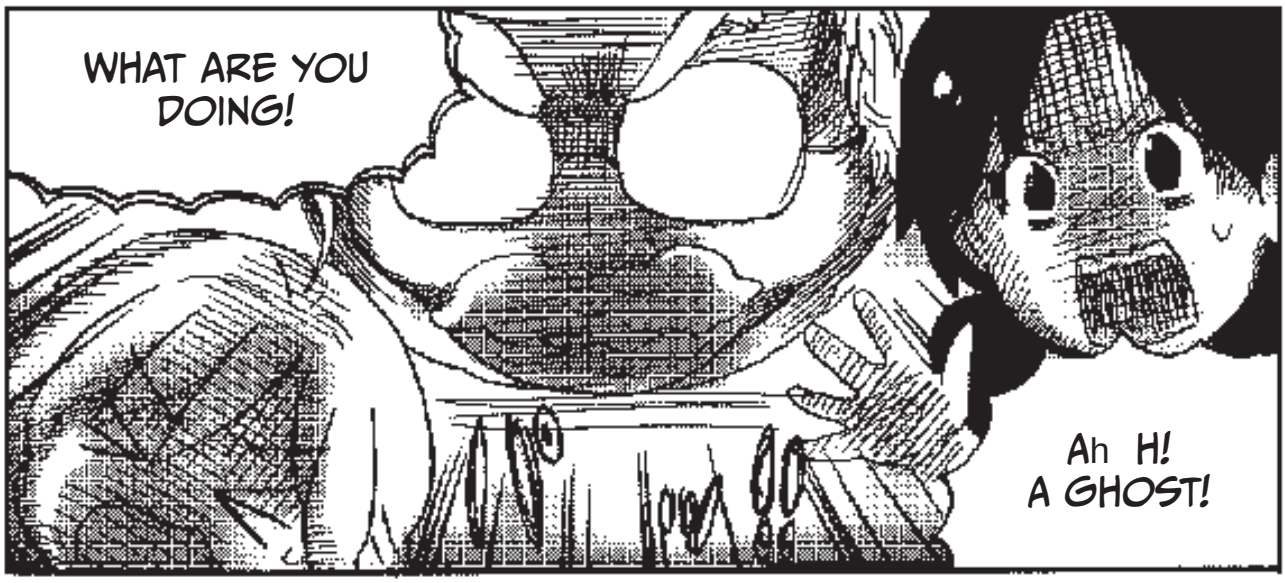

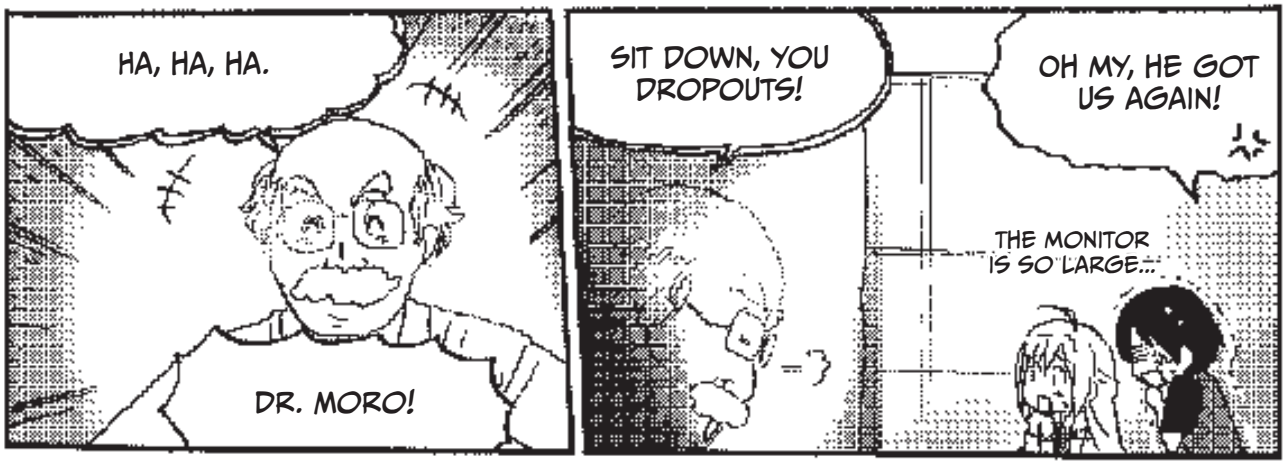

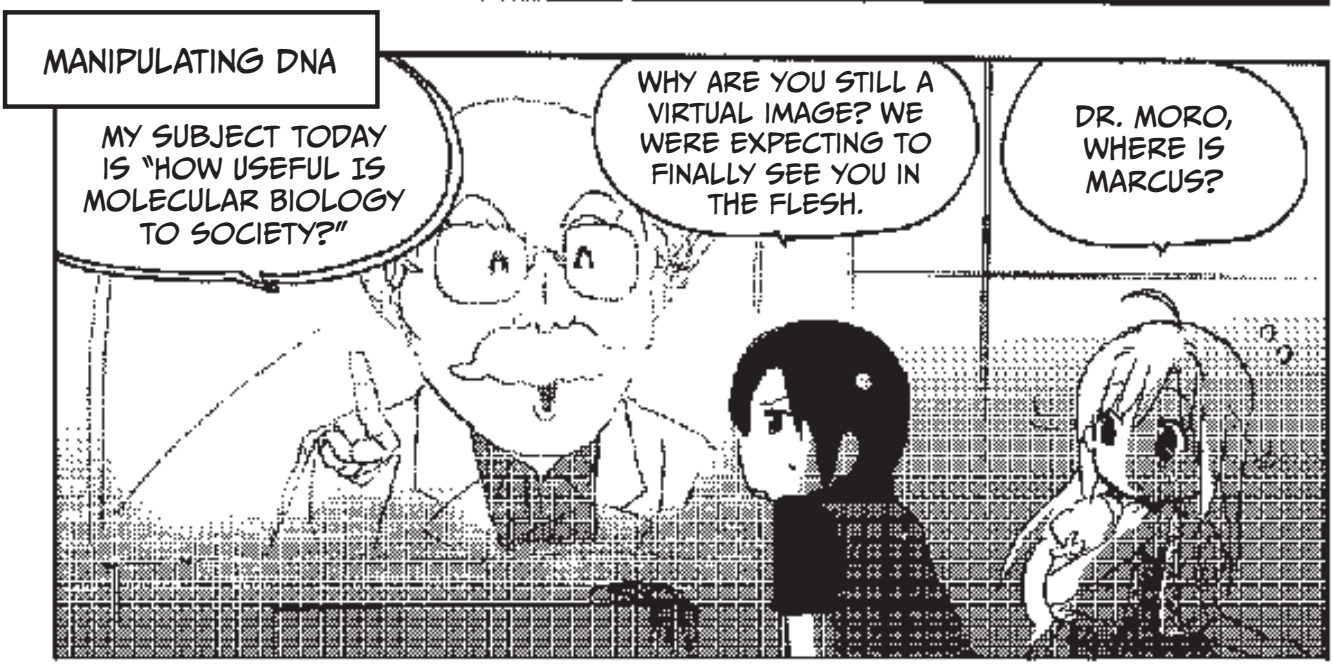

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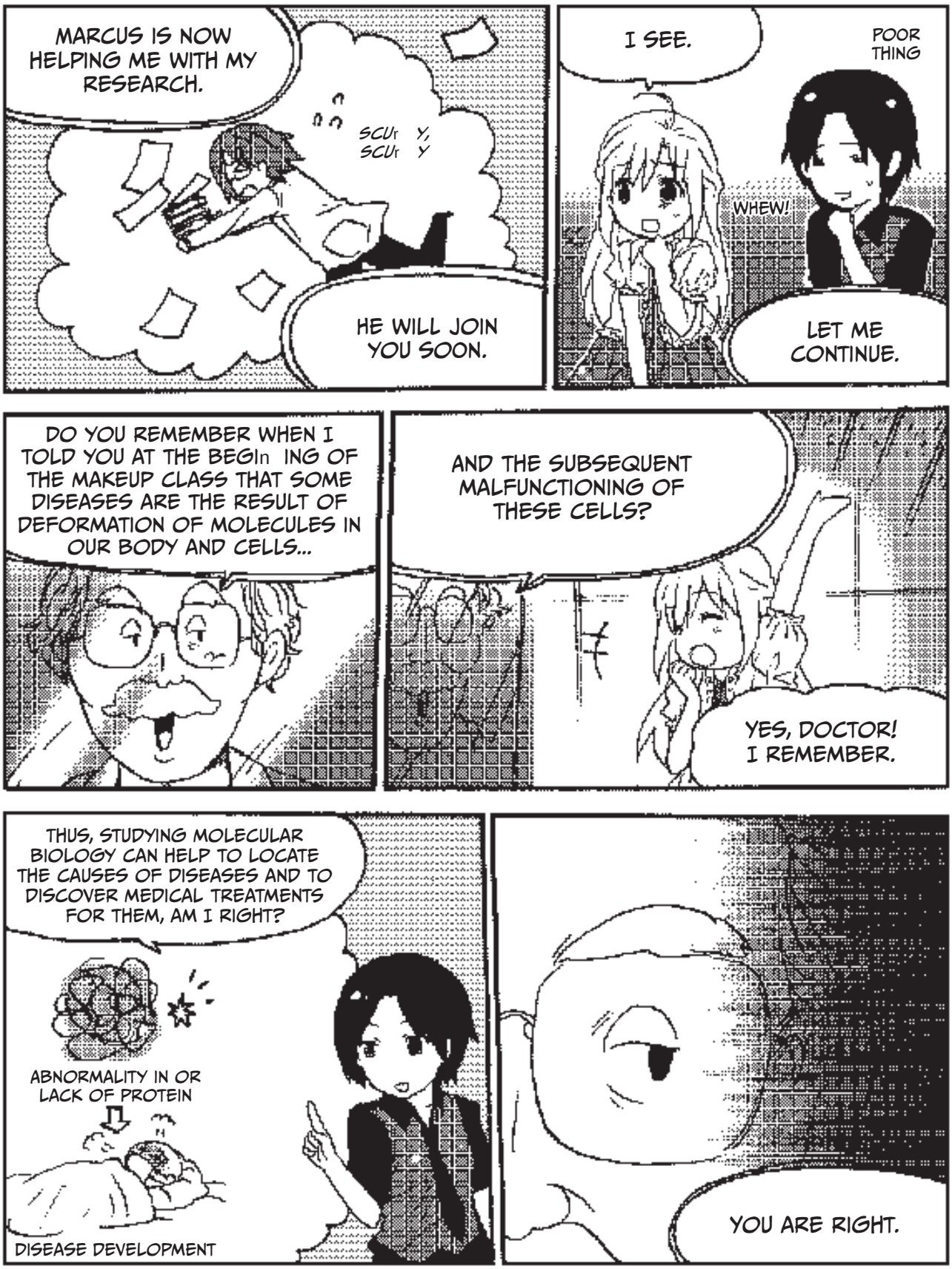

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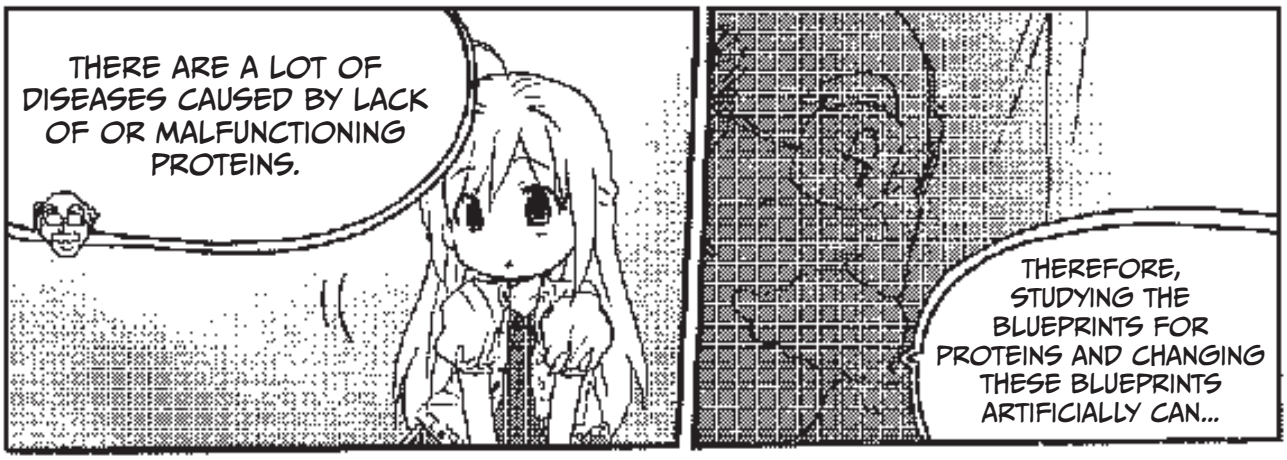
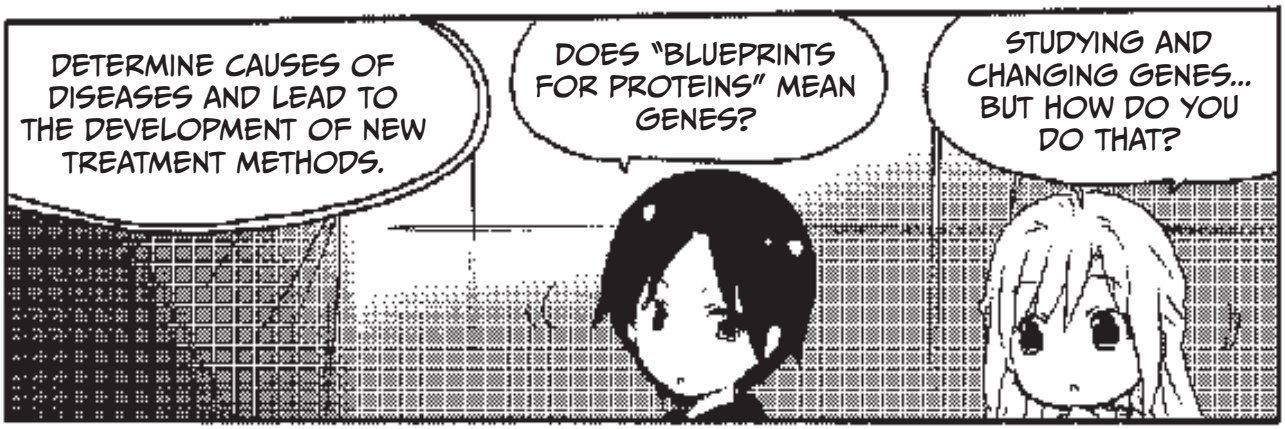
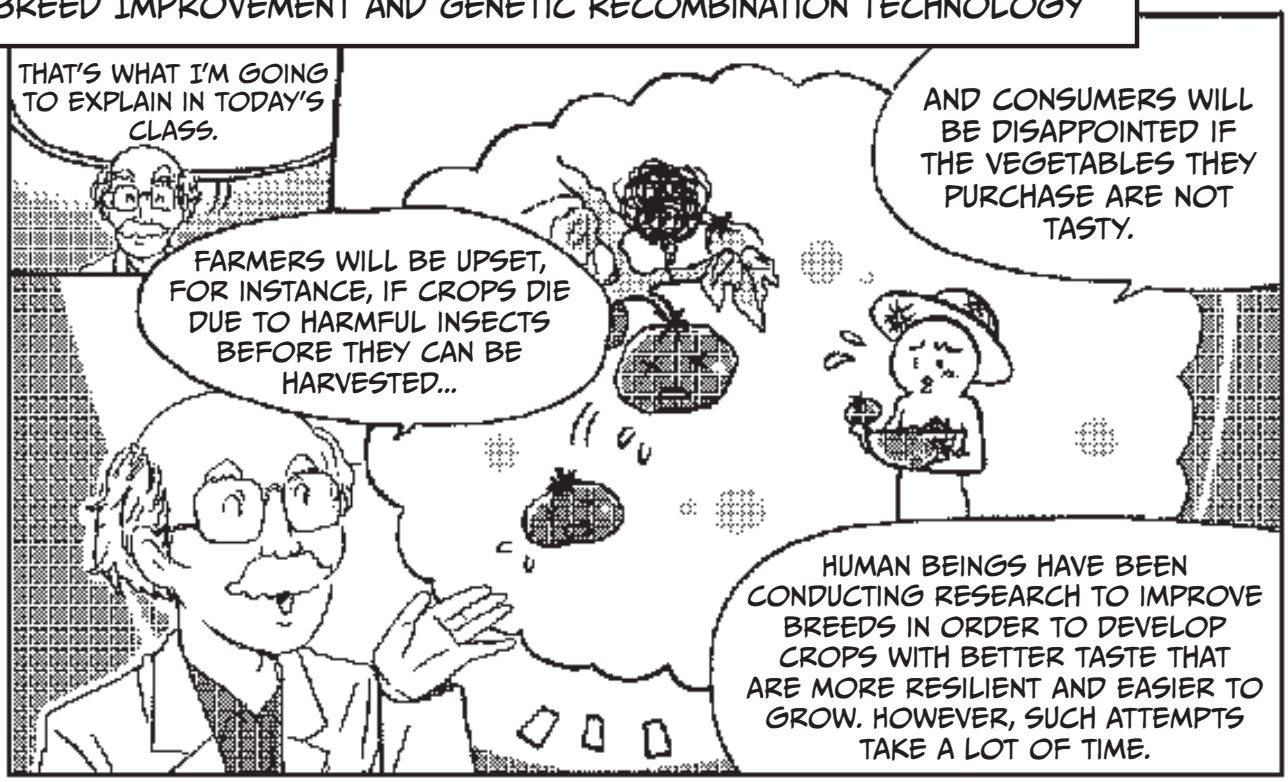




BREED IMPROVEMENT AND GENETIC RECOMBINATION TECHNOLOGY



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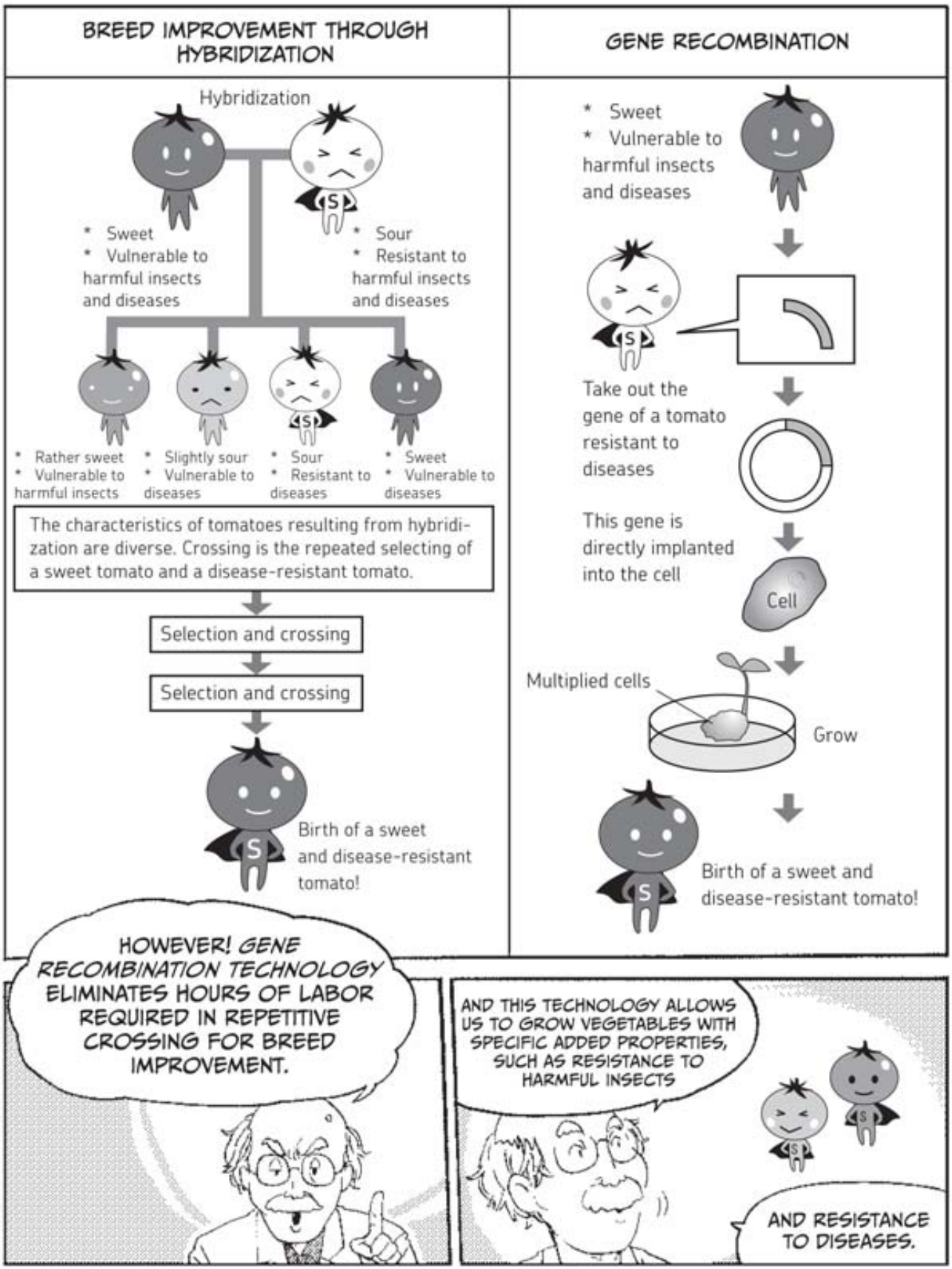

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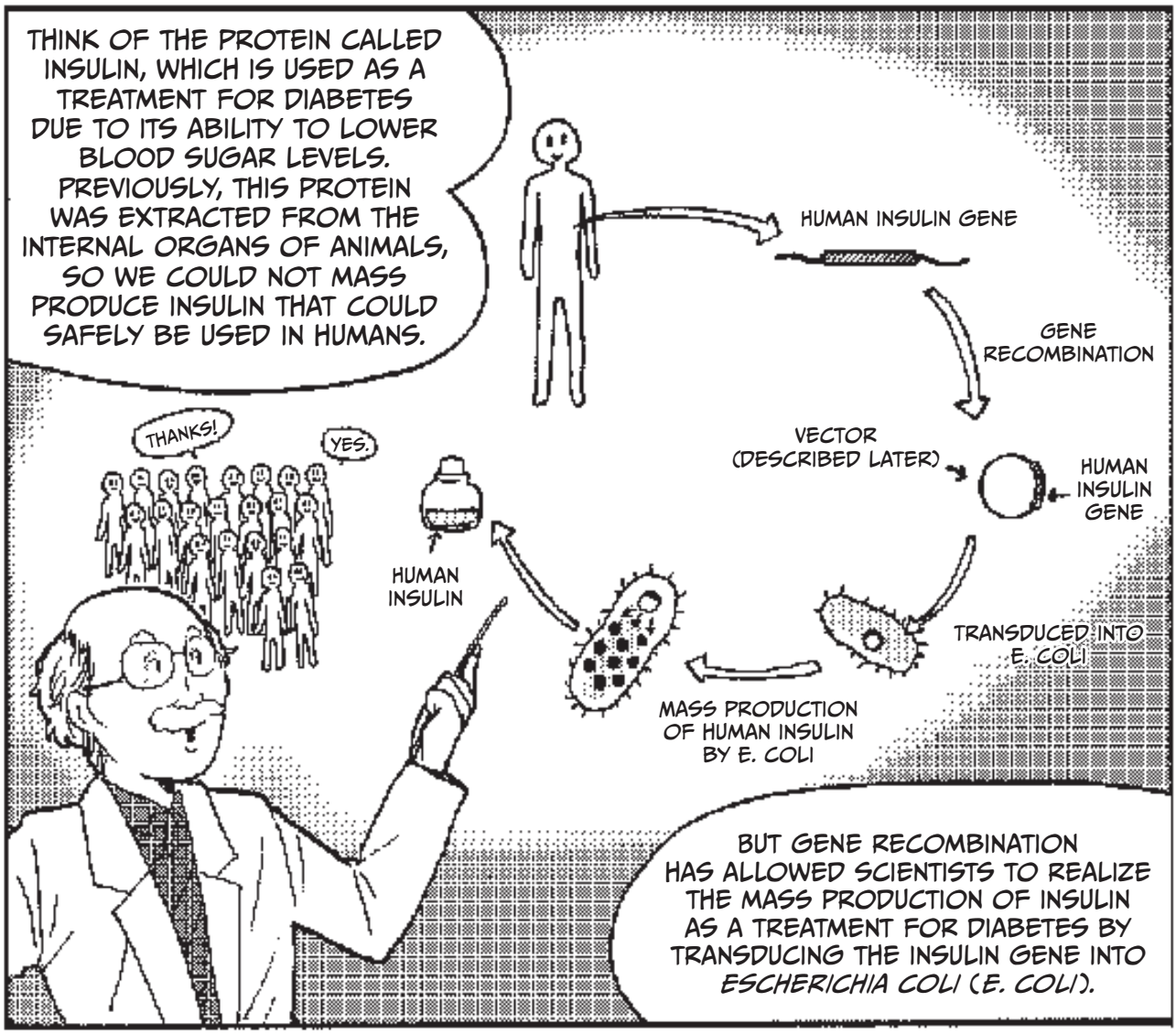

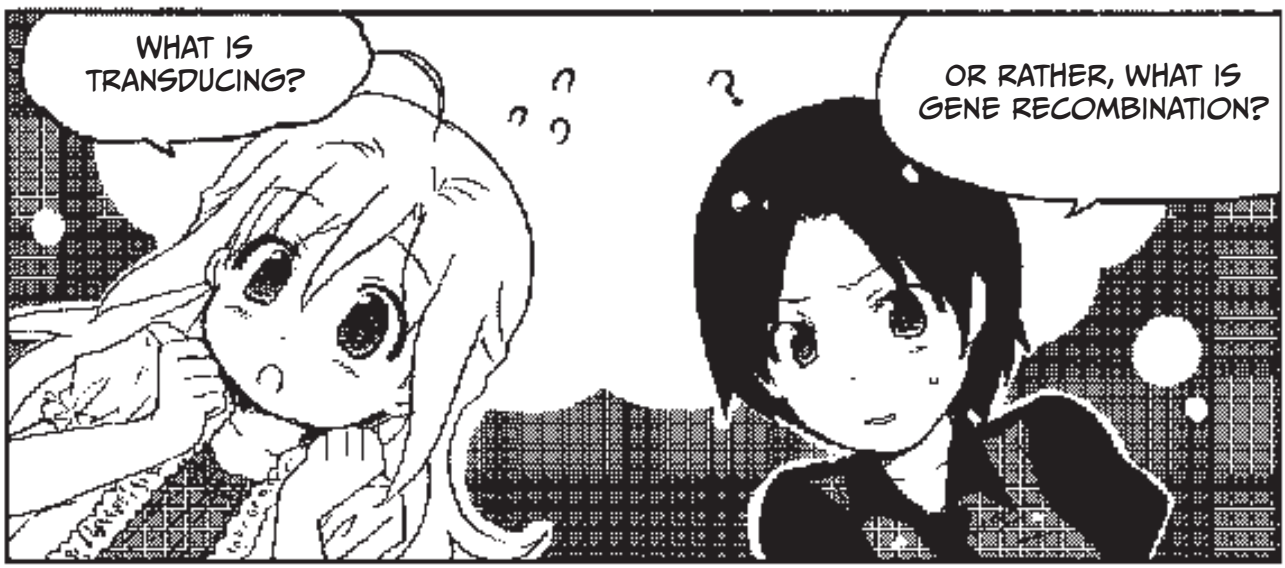

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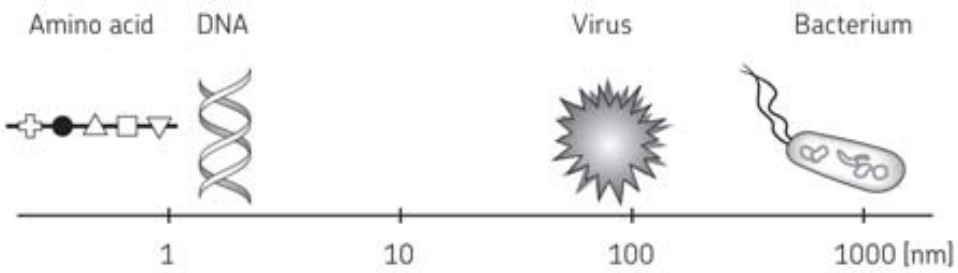
AN EXAMPLE OF GENETIC RECOMBINATION TECHNOLOGY

STEP 1: TARGET GENE IS MULTIPLIED

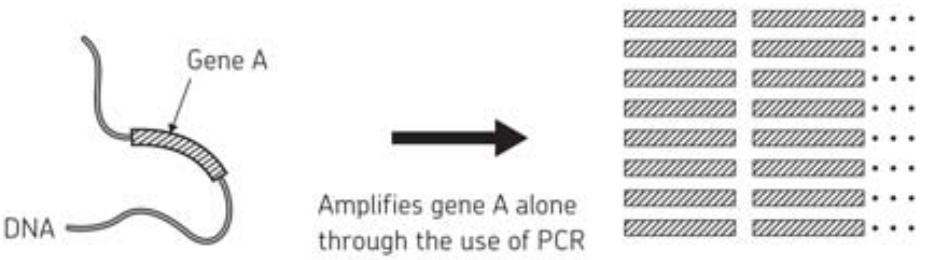
The width of a molecule forming the double helix of DNA is merely two nanometers (nm). One nm is one billionth of a meter (one millionth mm). DNA, the true identifier of genes, is so small that we cannot see it with our eyes.



You know, it is very hard to handle invisible objects. So how can we make it visible?

We talked about water at the beginning of this book. We can't see individual water molecules. However, when a vast number of water molecules get together, we can recognize the liquid as water. This also holds true for DNA. If individual DNA is not visible, why shouldn't we multiply it until it becomes visible?

One of the technologies used for that purpose is *PCR* (*polymerase chain reaction*). PCR is a process that allows scientists to multiply a specific gene in a sample of DNA. Using this technology allows us, for instance, to make millions of copies of one gene and purify the DNA from that gene alone, or to detect the presence of the gene in a sample (for more information about PCR and how it works, see page 203).



Amplification of genes

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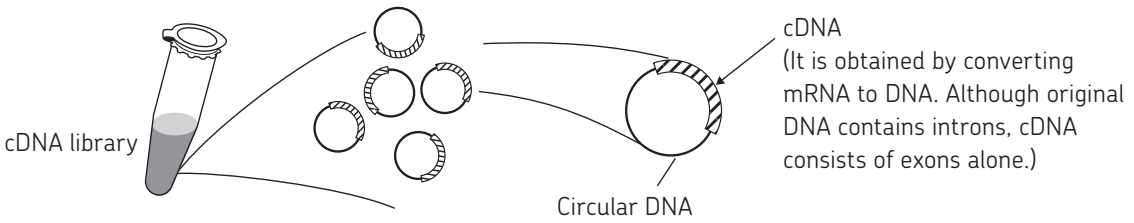
Researchers can now obtain genes from a large number of animals and plants (humans too!) using a database called a *cDNA library*. The genes are dissolved in a small volume of solution, which contains the cDNA of gene A. PCR works by using an RNA primer, a set of enzymes, and a pool of free nucleotides to make many copies of (amplify) gene A. PCR works differently than the cell's DNA replication process but uses many of the same raw materials.



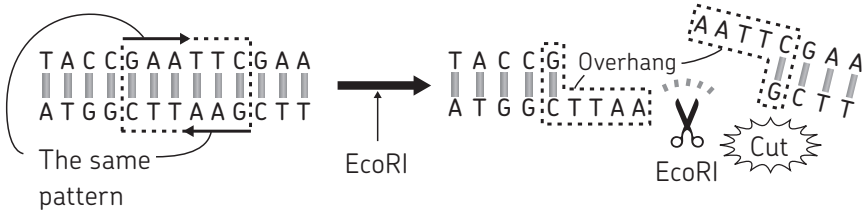
STEP 2: GENETIC RECOMBINATION—CUT AND PASTE

Another kind of technology is required to insert the amplified gene A into a new strand of DNA. This technique makes up the major part of gene recombination technology.

Its principle is *cut and paste*. It's similar to the well known computer task of inserting a sentence or word at another location. In the first step, you cut gene A away from the rest of the DNA using a special enzyme called a *restriction enzyme*. But as gene A comes away, there is a little *overhang* or area of overlap on each side.

Overlap? What do you mean by an area of overlap?

Restriction enzymes act like scissors, cutting the strands of DNA at a given sequence of base pairs. For example, a restriction enzyme called EcoRI cuts only the portion where the base sequence is GAATTC. Look at the following figure carefully and you'll find that the base sequence of the partner of the double helix is also GAATTC when read backward. EcoRI cuts this portion in a zigzag manner, leaving the sequence AATT without any pairs, which creates an area of overlap. These overhangs are sometimes called *sticky ends*.



One neat thing about PCR is that, when you are amplifying gene A, you can simply add on the DNA sequence recognized by the restriction enzyme to both ends of the gene. As gene A amplifies, this new sequence gets copied over, along with the DNA for gene A (for details, see page 203).

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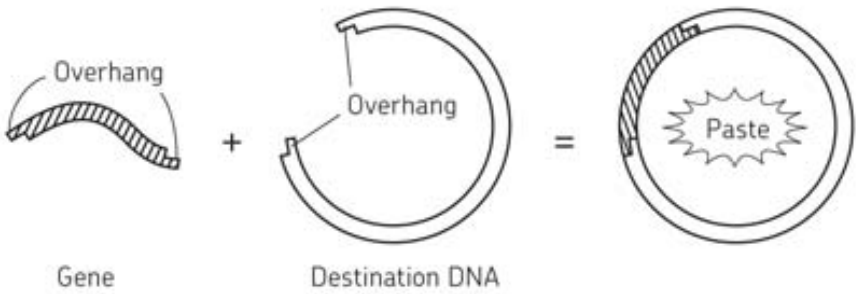
Using the same restriction enzyme, this technology cuts both the ends of the DNA for gene A, as well as the DNA within the target cell, and leaves the same area of overlap on each piece. In this way, you can paste gene A into the DNA in the target cell.

Then PCR mixes the two types of DNA (gene and destination DNA) and connects the overhangs with an enzyme called *DNA ligase*. This completes the pasting process.



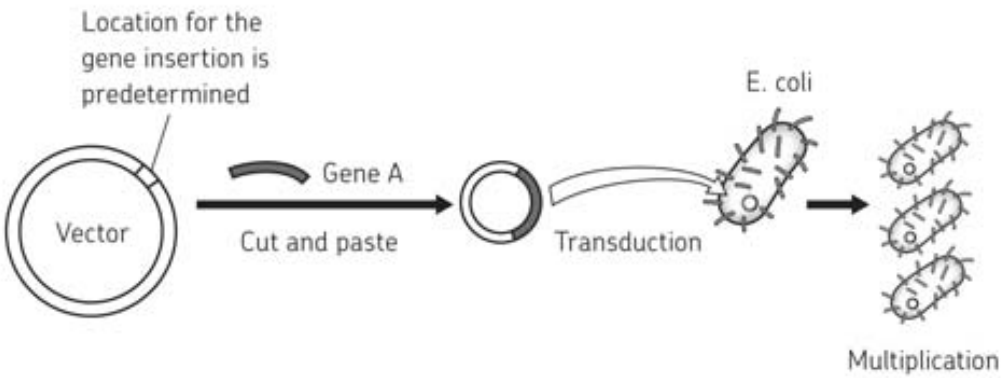
STEP 3: TRANSDUCTION AND CLONING

I've mentioned *destination DNA* several times. Why do we have to insert gene A into another DNA?

When inserting a gene into a living organism and having it implement its function (express the gene), you must insert it into a special carrier (made of DNA) through the use of the above cut and paste method.

This carrier is called a *vector*. It is made of circular or hoop-shaped DNA that was derived from a DNA called *plasmid* contained in the cells of bacteria such as E. coli. Researchers modified the plasmid, and a variety of vectors have been developed to serve various purposes (some vectors are derived from viruses).

Plasmids replicate arbitrarily in the cells of bacteria. Thus, if you transduce (there are several transduction methods, including the electric shock method) the plasmid-derived vector being inserted with gene A into bacteria such as *Escherichia coli* (*E. coli*)—which can easily multiply in a laboratory environment—you can easily create a vast number of them.



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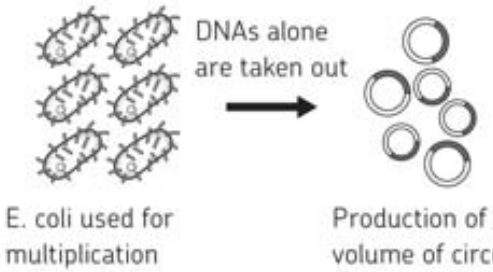
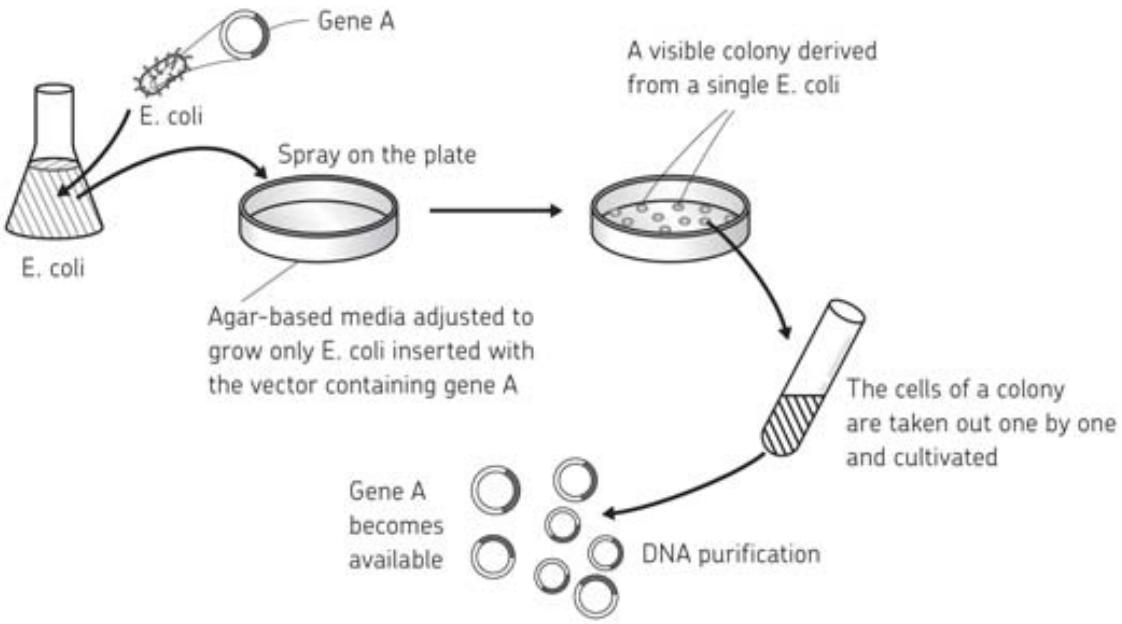
After the bacteria have multiplied many times, DNA purification allows us to obtain a massive number of copies of the plasmid (called *clones*) containing gene A. This process is called *cloning*.





We can get a massive volume of protein A by increasing the number of E. coli to cultivate protein A from the transduced gene A. In this case, a vector is designed to enable E. coli to thrive and transcribe a lot of gene A under very specific conditions, such as only when a certain antibiotic is present in the culture solution.

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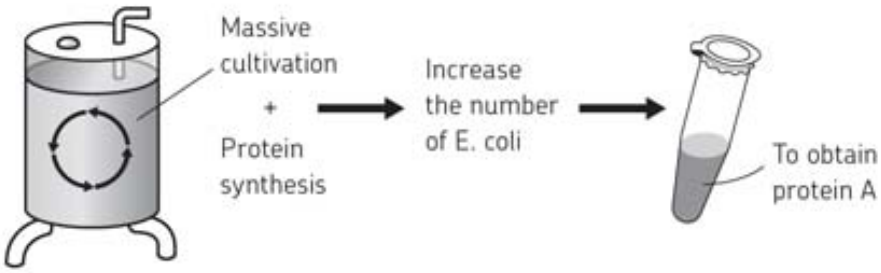

Currently, it is possible to construct proteins not only from bacteria such as E. coli, but also from various types of cells, including insect cells and cells of mammals. Vectors specialized for each are being developed.

METHODS FOR DETECTING AND ISOLATING DNA

A little while ago, we learned that as chromosomes condense they become visible under the microscope. But when we are working with just one gene, how can we see it? We can visualize the products of PCR, or any mixture of DNA, using a process called *gel electrophoresis*. This process manipulates many of the chemical properties of DNA to separate, purify, and visualize the DNA that we isolate and synthesize (using PCR) in the lab.

A solution containing a mixture of DNA is poured into holes (wells) at the top of an agar-based plate. The agar medium is made of strands of protein that act like mesh. As an electric current is run through the plate, the pieces of DNA move through the agar based on their size. Longer strands of DNA get stuck in the mesh of the agar and migrate more slowly than shorter strands. Why do we use an electric current? The sugar-phosphate backbone of DNA is negatively charged; as the current is applied the negative charge of the DNA is attracted to the positive pole.

Then we can use chemicals to actually *see* the DNA. Certain molecules can bind in between the nitrogenous bases of DNA. The chemical structure of these molecules makes them fluoresce under ultraviolet light. Adding this kind of chemical (ethidium bromide, for example) allows us to see DNA by having it fluoresce under UV light. In this manner, we can use gel electrophoresis not only to separate but also to see the pieces of DNA in a mixture.

If you have run PCR for a specific gene, you can simply cut out the bands of DNA on the gel that correspond to the gene you want (that is, with the same length as your gene). A pure sample of DNA can be obtained by dissolving the agar, extracting out the DNA using alcohol, and washing the sample clean. In this way, you can obtain a large volume of *pure DNA* (not containing DNA strands with a different base sequence or length) that can be used for other purposes, such as gene recombination.

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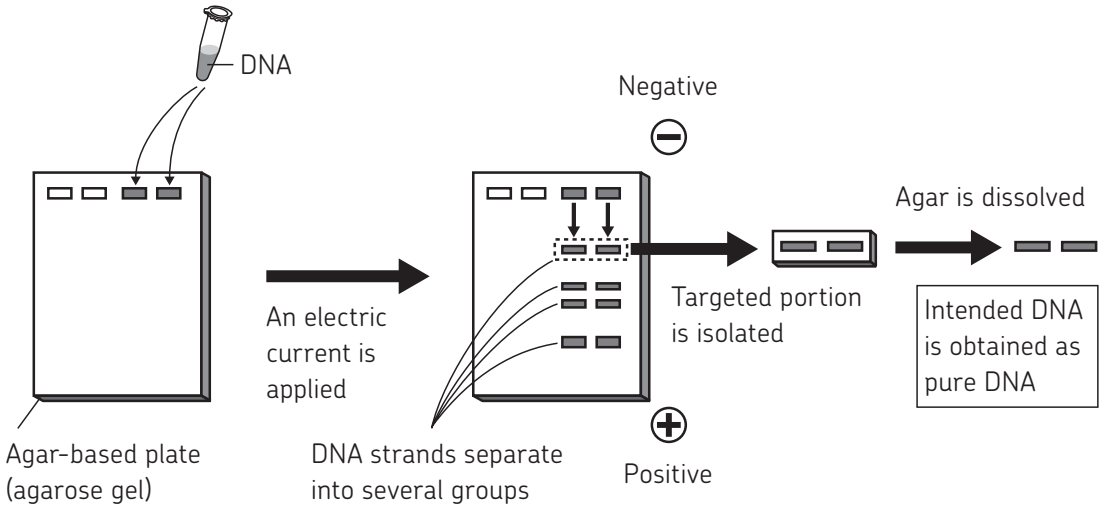

TRANSGENIC ANIMALS (KNOCKOUT MOUSE)

While gene recombination technology is applied in the improvement of agricultural crops and mass production of medicines, the number of areas it can be applied in does not stop there.

First of all, it is contributing greatly to the study of molecular biology itself. For instance, by transducing a certain gene into a cultured cell and analyzing what happens—namely, how the cell changes—

We can determine the properties of the protein constructed by the gene?

That's right! Genetic recombination technologies allow us to discover the function (or functions) of a particular protein. Moreover, with slightly more sophisticated techniques, you can study how cells behave once a gene is *deleted* to give you even more information about how a protein works in the cell. These methods are not limited to cell culture alone; they can also be used to make *transgenic animals*. Transgenic animals have an extra gene *added to* or *deleted from* their genome. They are very useful in studying how genes affect growth or disease development in a whole organism (mice are the most common, but fruit flies, rabbits, zebrafish, yeast, and mustard plants are also used).

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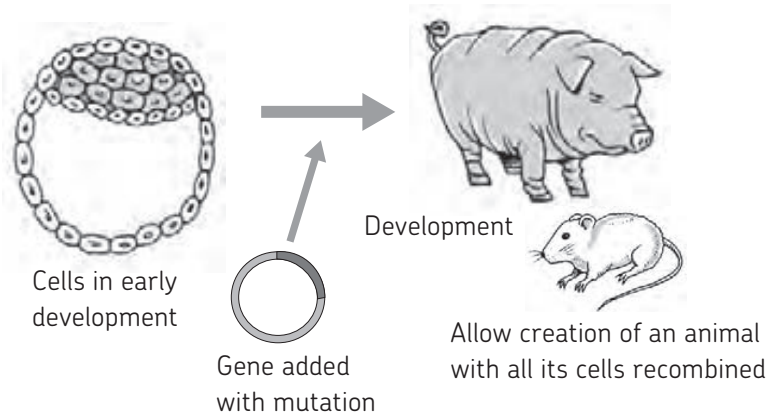

By using gene transduction methods on animal cells at a very early stage of development, you can incorporate a new gene into some, but not all, of the cells of the developing animal. This makes an animal with a mixed genome, called a *mosaic*. By breeding the offspring of mosaic mice together (some eggs and sperm will have the new gene, called a *transgene*) you can create animals that have the transgene as part of their DNA, so every cell will express that new gene.

By transducing a gene into an *ES cell* that is mutated or a gene sequence that deactivates another gene (already in the mouse's genome), you can create transgenic animals that lack a certain gene (and thus a functioning copy of that protein), called *knockout mice*.

What is an ES cell?

An ES cell is an *embryonic stem cell*. It is a very special kind of cell that is harvested from an embryo before any of the cells have started to specialize into one tissue or another. Because an ES cell has the ability to develop into a cell in any kind of tissue in the body, it is called *totipotent*. If you add in or knock out a gene in an embryonic stem cell and then put it back into the growing embryo, the mouse will become a mosaic.

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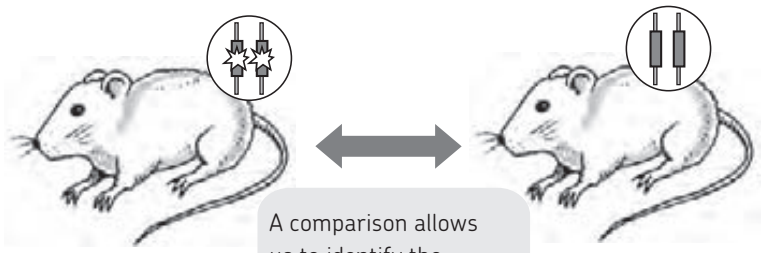
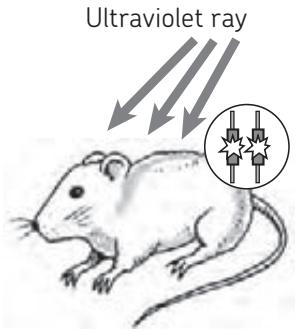


Knockout mouse

A comparison allows us to identify the function of the mutated gene that was inserted.

Normal mouse



Knockout mouse

If, for example, a knockout mouse exposed to ultraviolet becomes more prone to cancer, we may say that the subject gene has the function of preventing the development of cancer when exposed to ultraviolet rays.

Knockout mice are particularly useful. We can determine the original function of a gene by studying the differences between a knockout mouse and a normal mouse. For example, if a knockout mouse exposed to ultraviolet light develops more skin cancer than a normal (*wild-type*) mouse, we may say that the target gene likely helps prevent the development of skin cancer caused by UV rays. Note that we would need further studies to show *how* the gene prevents skin cancer. The 2007 Nobel Prize in Physiology and Medicine was awarded to the three scientists who discovered the method for producing knockout mice using ES cells.

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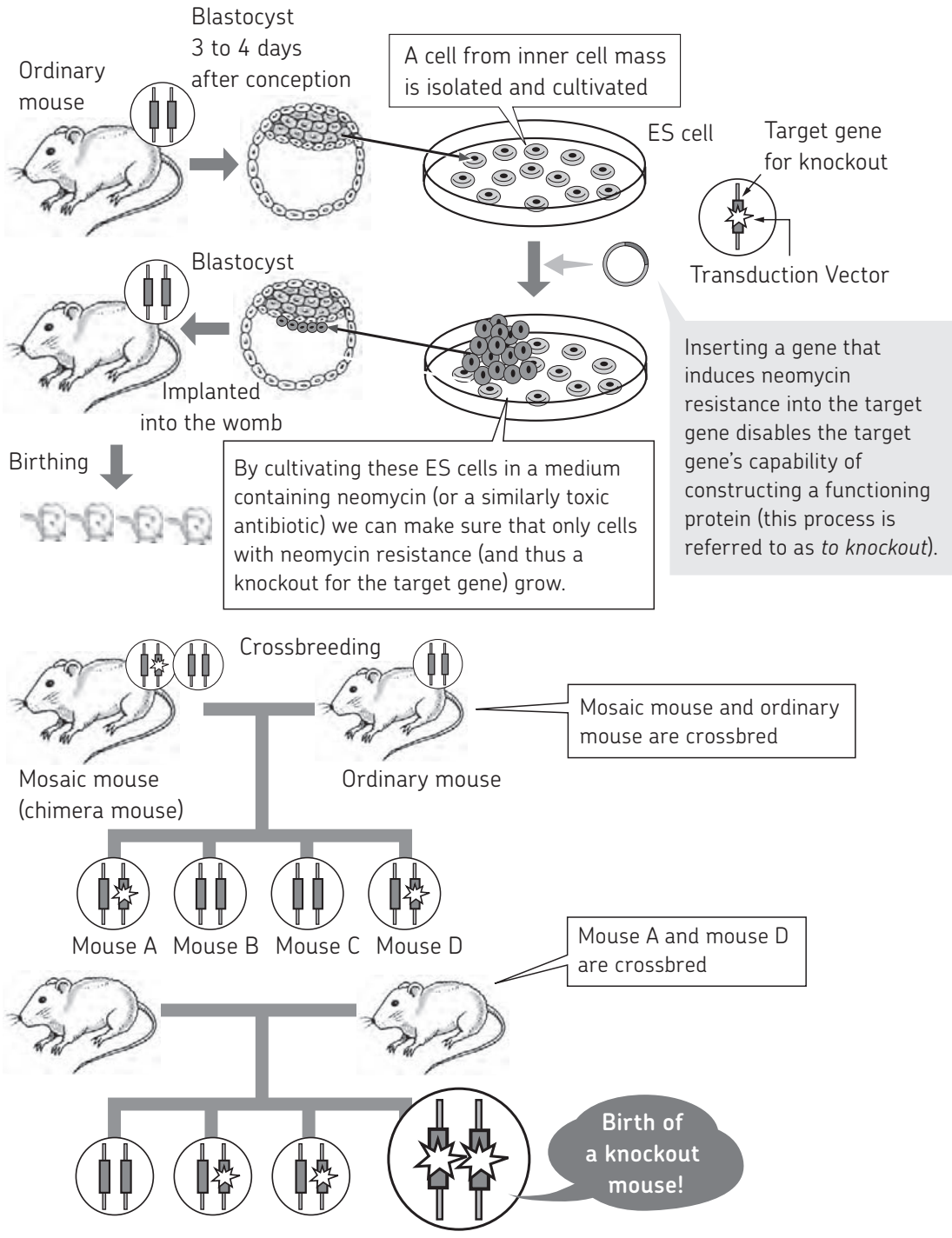

The birth of a knockout mouse (an ES cell-based example)

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PERSONALIZED MEDICINE AND GENE THERAPY: ARE GENETICS THE FUTURE OF DISEASE PREVENTION?

Have you ever heard the term *metabolic syndrome*?

My father has metabolic syndrome.

Wait, wait, are you saying your father has the syndrome just because his waistline is large? That is wrong.

Oh, why?

Metabolic syndrome refers to the state where someone with visceral fat obesity has developed two or more of the following: a high blood sugar level, high blood pressure, or dyslipidemia. Diseases like high blood pressure and dyslipidemia are called *lifestyle-related diseases* and are considered to be the result of lifestyle, including dietary habits and exercise. Lifestyle-related diseases include serious ailments such as diabetes, cardiac infarction, brain infarction, and cancer of the large intestine, which in some cases can result in death.

I think inadequate exercise, as well as excessive eating and drinking, is the cause.

I agree, but there is evidence that some of these lifestyle-related diseases are also caused by genetic factors.

Genetic factors?

Demographic studies, that is, studies looking at a whole population, have shown that mutations in certain genes make a person much more likely to develop lifestyle-related diseases independent of their diet and exercise level.

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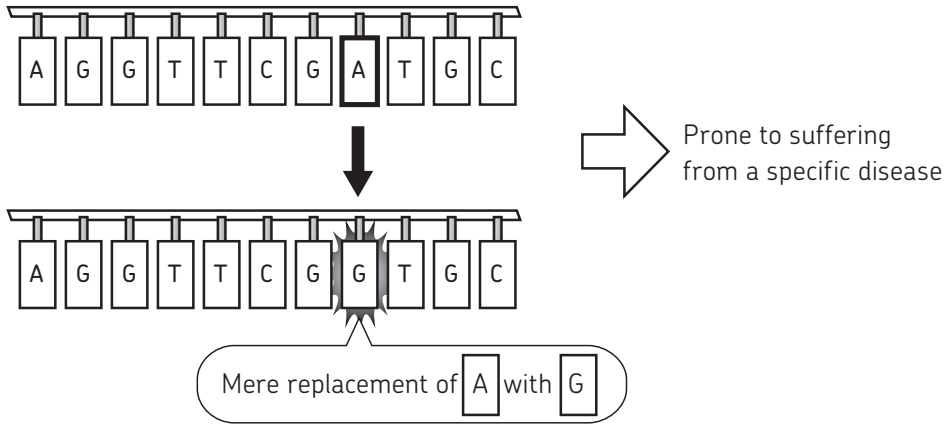
I see, so for conditions like metabolic syndrome it's not just someone's lifestyle that is the cause.

That's right. And a change in the gene often involves the replacement of only one base in the DNA sequence with another. The interesting thing is that the proteins still work close to normal, but slight changes in the structure can change how the protein works in a subtle way. There is now evidence that such small replacements, over the course of a lifetime, can increase the risk of suffering a heart attack or getting certain types of cancer.



Note: This is a conceptual diagram and does not indicate that this base sequence tends to cause a specific disease.

Thanks to recent research, our knowledge of how genes can contribute to disease is growing. In the future, you may be able to have your own genome sequenced to look for diseases you might be at risk of developing. Physicians call this concept *personalized medicine*, tailoring your medical treatment to your known risk of diseases based on your genes.

Knowing which diseases I am at risk for—that's scary. I would rather not know.

I would want to know! Because then I would be able to take all necessary measures.

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Necessary measures?

You know, just so I could prepare mentally.

In addition to mental preparedness, you would be able to make changes to your lifestyle. By knowing ahead of time what diseases you are at risp for, you can tailor your diet, how much you exercise, and what activities you do to help delay the onset of these preventable diseases. Personalized medicine may one day be a cornerstone of preventative health care.

That's right, change my diet and exercise more. That's what I meant!

I guess that makes sense . . .

GENE THERAPY

There is another frontier in medical treatment that you should know about. This new treatment is called *gene therapy*.

I've heard of gene therapy. I think it was on the news.

What kind of treatment is it?

Imagine that a baby is born with an abnormal gene and that gene is important for life. With a serious genetic disease, the baby could die at birth or before it ever gets a chance to grow.

In order to save the lives of people with serious genetic diseases, contemporary medicine sometimes employs an approach called gene therapy. Normal, healthy, genes are inserted into specialized vectors that are artificially transduced into some of the cells of the sick person. Using this method, the normal version of the gene is added back into the body so that cells can make a functioning, normal version of the missing or nonfunctional protein.

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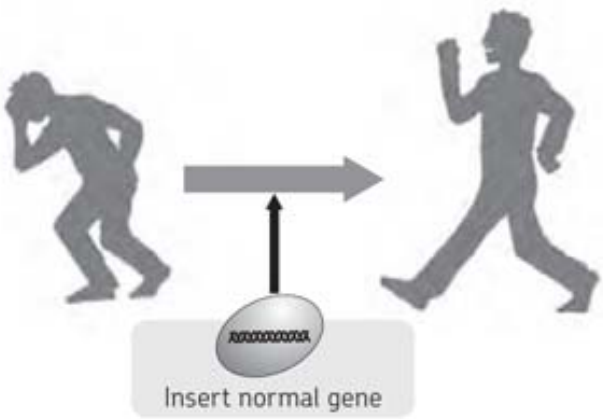
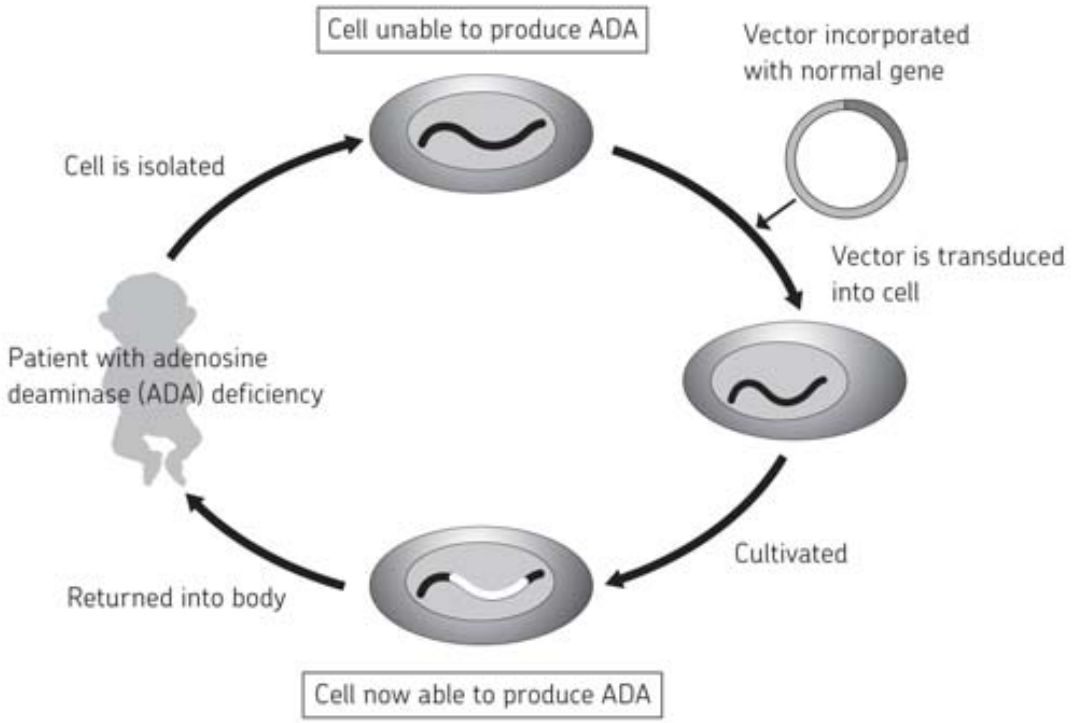


Gene therapy was first successfully used to treat a disease called *adenosine deaminase deficiency*. People with this disease are born without the ADA gene. The ADA protein is used in the metabolism of nucleic acids (DNA and RNA). The treatment injected a normal ADA gene into a patient with adenosine deaminase deficiency, giving the patient back the ability to break down DNA and RNA, curing the disease.



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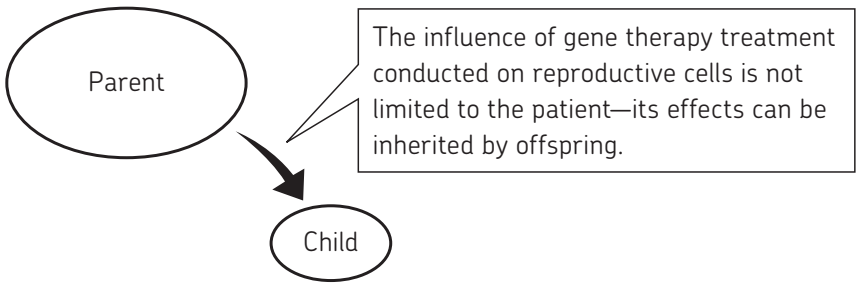
It seems like gene therapy is a dream come true for people with genetic diseases.

Sadly, things are not so simple.

Why not?

Gene therapy is very difficult technically, often dangerous, and still experimental. Genetic diseases where gene therapy could work well, like ADA deficiency, are rare. While some trials of gene therapy have been used on patients with brain tumors and breast cancer, the trials have had limited success. Additionally, there are many ethical complications that have stopped gene therapy from coming into widespread use. There are restrictions on the handling of genetic material and on what tissues are candidates for gene insertion. There are other ethical considerations involved in testing experimental therapies on children.



Because developing gene therapy treatments requires a lot of money and manpower, its usage is mostly limited to diseases that are otherwise incurable.

But . . .

I know what you're going to say. How can we forget about all the patients who would benefit from further study of gene therapy? We aren't ignoring them. It's just that gene therapy, like any other new technology, has limitations.

It seems like a difficult problem.

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THE RNA RENAISSANCE

Scientists believe that, long ago, RNA was the first and, at that time, *only* nucleic acid. Well before human life existed on earth, RNA carried out the role of encoding genes and passing them on to future generations. At some point, RNA's role was replaced by DNA, a much more stable molecule for storing genetic information. As researchers began learning about molecular biology, they thought that protein synthesis was the only important function that RNA had.

But recent discoveries suggest that RNA has many different and important functions. On top of that, scientists are becoming experts at manipulating RNA to do even more jobs within the cell. In the current world of molecular biology, studying and manipulating RNA is more in fashion than studying DNA. Some researchers refer to our expanding knowledge and experimentation with RNA as the *RNA renaissance*. RNA, which until recently was thought of as just a copy, has captured the imagination of scientists all over the world.

RNA INTERFERENCE: USING RNA TO ALTER GENE EXPRESSION

The 2006 Nobel Prize in Physiology and Medicine was awarded to two American molecular biologists—Andrew Z. Fire and Craig C. Mello—who discovered a phenomenon called *RNA interference*. A very short, complementary strand of RNA can bind to a molecule of mRNA in the cell. This binding creates a double-stranded molecule of RNA, which the body recognizes as foreign. Enzymes in the cytoplasm then attack and break down the mRNA and interfering RNA, thus stopping that mRNA from being made into a protein.

Why on earth was the Nobel Prize was awarded for discovering such a strange and destructive process?

Although this interference is destructive to mRNA, it is convenient from the viewpoint of the entire cell. Breaking down mRNA is one way of suppressing that gene's expression, which helps maintain the delicate balance of proteins in the cell. It was originally thought that the interfering short RNA strands were a mechanism to defend the cell against viruses, but recent studies have determined that too much

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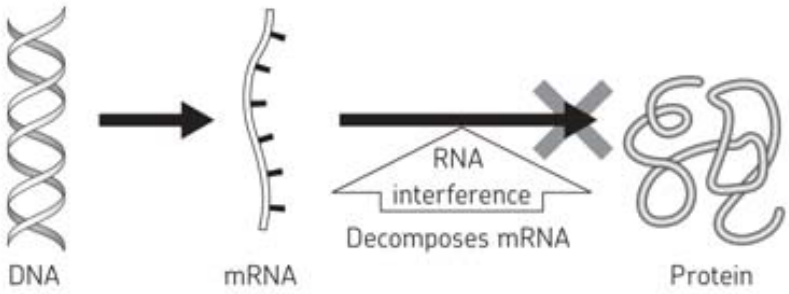
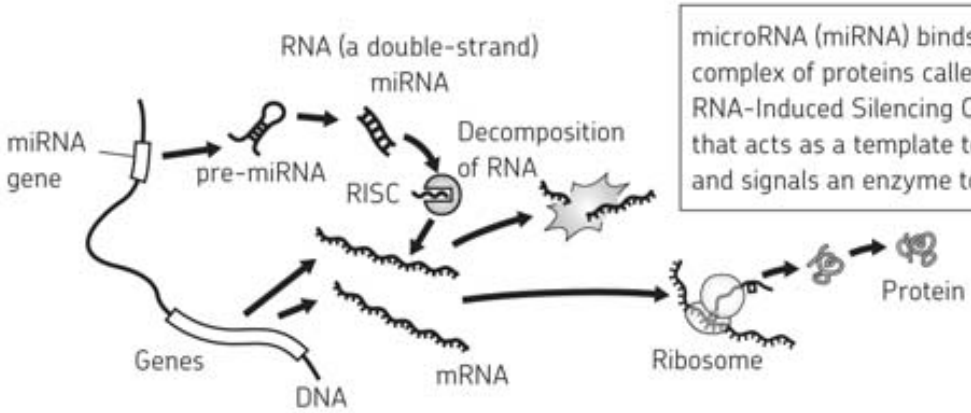


The small strand of RNA disables protein synthesis!

of this short interfering RNA exists for this to be its only role. This process appears to have emerged from the cells' defense mechanism against viruses. Viruses are the only organisms that still use double-stranded RNA to encode their genes, and the cell has many enzymes to break down double-stranded RNA. Studies have shown that a vast number of such "interfering" short-strand RNAs exist in our cells, apart from any viruses.

Any functioning society must have balance. Similarly, your cells must also have balance. This happens through the control of gene expression. RNA, a nucleic acid, is one factor that contributes to the balance of gene expression.



microRNA (miRNA) binds to a complex of proteins called the RNA-Induced Silencing Complex (RISC) that acts as a template to bind mRNA and signals an enzyme to destroy it.

As you can see, RNA has many functions. We had already learned about mRNA, tRNA, and ribosomal RNA used in making proteins. Now we also know about another function of RNA. RNA can be used to alter gene expression using *short interfering RNA* (*siRNA*) and *microRNA* (*miRNA*). Other kinds of RNA act on their own as enzymes, called *ribozymes*. It has been discovered that over 70 percent of the mouse genome can be used to transcribe RNA. Substantial portions of the human genome are also used to synthesize RNA. While scientists have made great strides in learning about the functions of RNA, there are still many kinds of RNA molecules whose functions remain a mystery.

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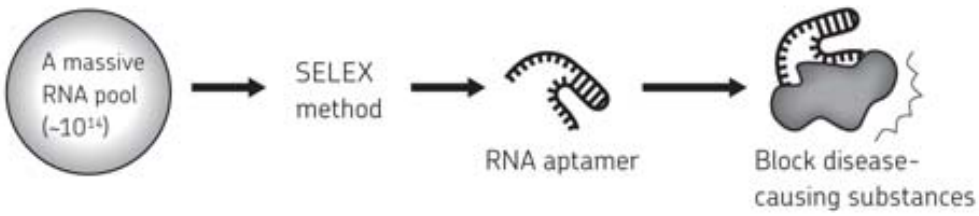
CAN RNA CURE DISEASES?

With all of the advances in RNA research, scientists have begun looking at how RNA might be used to treat diseases. Currently, many companies are working on drug discovery projects using siRNA. RNA has the following two features that make it amenable for drug development: RNA can be easily synthesized with any combination of base sequence (A, G, C, U), allowing for a large variety of shapes and sizes; and the cell can easily break down RNA, allowing for quick metabolism of any RNA-based drug.

Certain molecules of RNA have the ability to bind to proteins. RNA medications work by binding to abnormal, disease-causing proteins. The ability for a specific sequence of RNA to bind to a protein is analyzed and optimized using a procedure called the *SELEX method* (*Systematic Evaluation of Ligands by Exponential Enrichment*). Using this method, sets of RNA molecules that are very similar (but not the same) in structure are analyzed to see which best bind to a specific protein. RNA that is targeted to bind to a protein is called an *RNA aptamer*.



The first RNA-based medicine to be approved by the US FDA was *Pegapatnib*, an RNA aptamer that binds VEGF 165 and is used to treat age-related macular degeneration, a crippling eye disease. Because RNA and RNA-based medications are easily broken down, these medications are believed to have few side effects. Whether RNA aptamers will become miracle-drugs has yet to be seen and depends on the result of much more future research.

HOW EXACTLY DOES PCR WORK?

The method for multiplying, or amplifying, genes for use in the laboratory is called *PCR* (*Polymerase chain reaction*; see page 187). Let's examine more details of this method here. Remember from Chapter 3 that the protein (enzyme) that replicates DNA is called *DNA polymerase*. Using DNA polymerase, PCR multiplies a copy of a specific gene in an exponential manner.

This process happens in a very controlled manner in the body, but for use in the lab, PCR was designed to create continuous replication of a gene by altering the enzymes and the temperature of the reaction. A large pool of nucleotides and two RNA primers for the gene of interest (one for each strand, forward and backward) are added to a buffer containing a small sample of DNA. An increase in temperature

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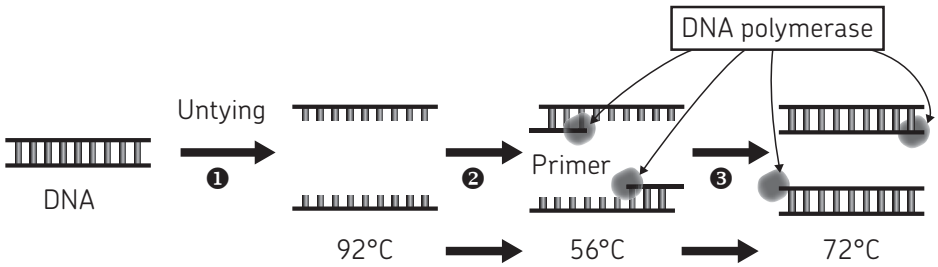
melts the DNA into two single strands; as the solution cools, the RNA primer can bind to the DNA and DNA polymerase makes a copy of the gene using the free nucleotides in the solution.



❶ 92°C: DNA is separated to a single strand at 92°C.
❷ 56°C: When cooled to 50°C, mating primer is bonded with each strand.
❸ 72°C: At 72°C, DNA polymerase starts DNA synthesis.

When the temperature is increased to 92°C again, two completed DNA strands separate from each other. Then, the steps are repeated over and over again.

Usually, proteins break down, or *denature*, at high temperatures (40–50°C or 100–125°F). A very special version of the DNA polymerase enzyme is used in PCR, one that can withstand very high temperatures. This enzyme was isolated from a bacterium called *Thermus aquaticus*. This bacteria is found only in hot springs and was discovered in the geyser pools of Yellowstone National Park, living in near-boiling (165°F) water.

Karry Mullis developed PCR in 1993 and won the Nobel Prize in Chemistry that same year. Since then, compact machines have been developed which can be programmed to control the temperature and time of each step of the PCR reaction to mass produce copies of a gene. As was mentioned earlier (page 188), adding additional base pairs to the primer incorporates this new sequence into the copied gene. It is in this manner that PCR can be used to create genes that can be cut and pasted into a plasmid with restriction enzymes.

Newer methods of PCR have been developed that allow you to turn RNA into DNA to be copied via PCR (*RT-PCR*) and to count the number of copies of DNA produced so accurately that you can use equations to figure out how many copies of a gene were present in a sample (*quantitative PCR*). These methods working together can help you figure out how much mRNA a cell is making of a particular gene (directly measure gene expression) and also make up the basis of DNA testing in forensic science.

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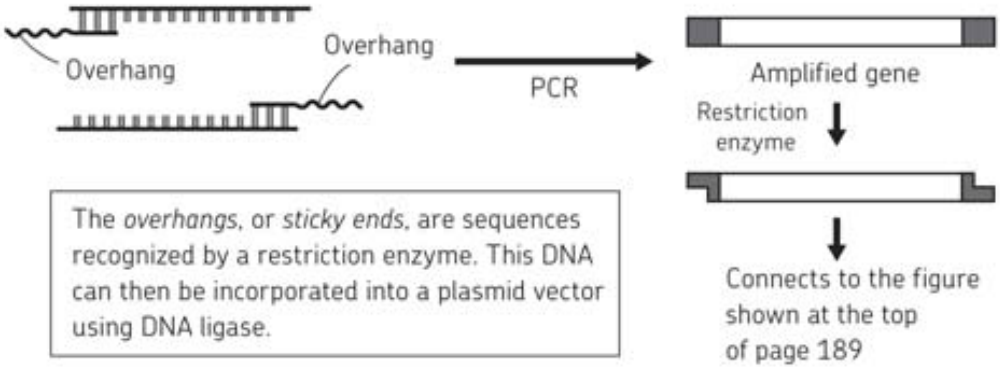
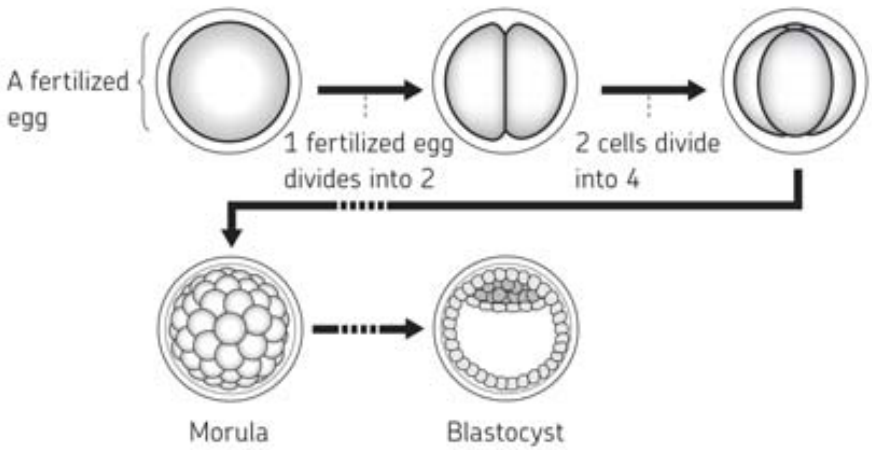


The *overhangs*, or *sticky ends*, are sequences recognized by a restriction enzyme. This DNA can then be incorporated into a plasmid vector using DNA ligase.

Connects to the figure shown at the top of page 189

HOW TO PRODUCE CLONED ANIMALS

Every animal, humans included, grows from a single fertilized egg. This single cell divides over and over again, growing into an embryo. Each of these young cells will develop into many different tissues, giving order and function to our body. In the figure below, you can see how repeated cell division turns a fertilized egg into an embryo.



We have learned a lot about how the study of genetics can be useful to mankind. For years, scientists have tried to develop methods to create plants and animals with the exact same genomes so that we can better study and understand genetics. This process is called *cloning*. The concepts of how it works are simple, but it took researchers years to perfect these methods.

How can we clone an animal? An embryo is removed from a female's womb at a very early stage and is broken up into individual cells. The nucleus from each cell is removed and then re-inserted into an unfertilized egg that has already had its nucleus removed. The eggs are bathed in chemical signaling molecules that convince the eggs that they have been fertilized. These eggs are then transplanted into the

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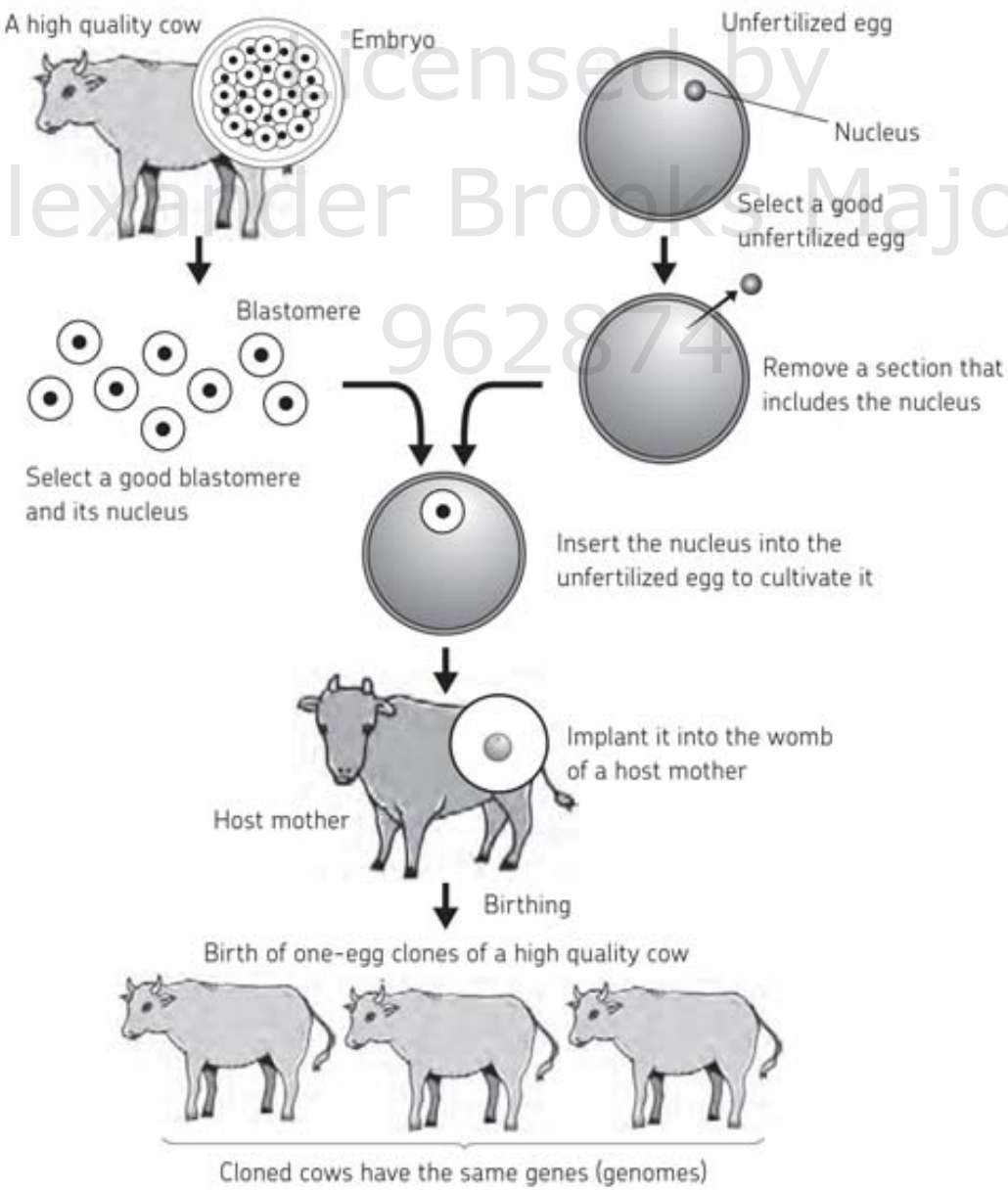
wombs of several other females. As a result, many individual organisms with the exact same genes (*genomes*) are born. Today, the agriculture industry uses this method to breed a large number of the same animal, or *clones*, with desirable traits. With just one fertilized egg, many cows (and a lot of beef, leather, and milk) can be produced.

This process may sound a little familiar. It is like identical twins, in that two individual organisms are born from a single fertilized egg. Animals cloned in this way have male and female parents, just like those produced from ordinary breeding. They are not the clones of their parents, but of each other.

How to produce cows cloned from a fertilized egg



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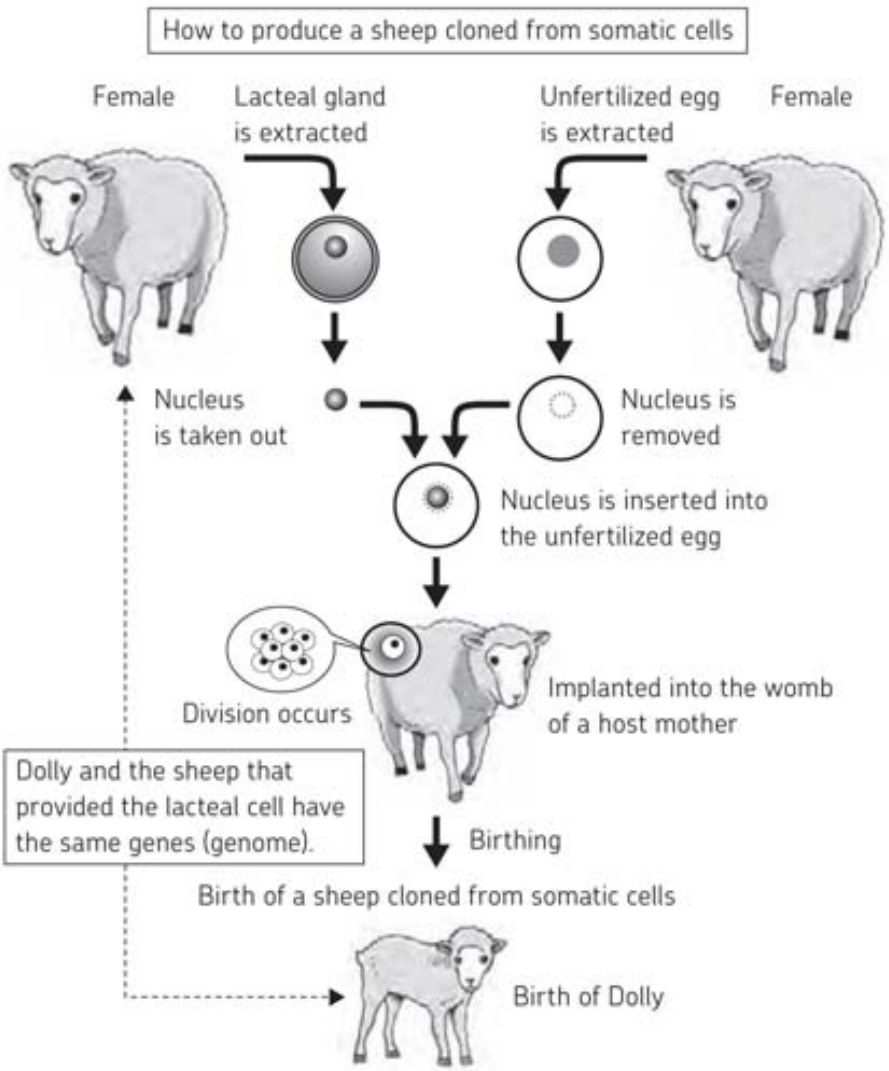
Scientists have developed more than one way to clone an animal. You may have heard of the sheep, Dolly, who was cloned in a British laboratory in 1996. Dolly was the first cloned mammal to have only one parent. Instead of being cloned from a sex cell (like the eggs mentioned above), Dolly was cloned from a *somatic cell*. Let's take a look at how this kind of cloning works. The process begins by removing the entire nucleus from a breast cell from the parent sheep (the cell was from the *lacteal gland*, an organ that excretes milk). An unfertilized egg is harvested, and the nucleus of the unfertilized egg is replaced with the full nucleus from the breast cell. The egg is then implanted into a host-mother sheep.



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The clone that is born has the exact same genome as its mother, as well as any siblings made in the same way. In this case, Dolly the sheep had no father, just a mother. After the success of Dolly, mice and cows have also been cloned with somatic cells.

Further advances in cloning technology may end up looking a lot like a science fiction novel. It may be possible to produce *cloned humans* using *nuclear transfer*, like Dolly. Theoretically, a person produced using this process will have the same genome as the person (parent) who provided the nucleus. But as we know from twins, people raised in different environments will turn out differently from each other. The idea of human cloning is fraught with legal and moral complications and, while many nations permit studying human stem cells, most nations ban research that would lead to cloning a human.

MOLECULAR EVOLUTION: HOW GENES CAN TELL A STORY

We normally think of evolution as a process that happens in nature. The environment around an organism, from predators to a changing climate, can shape how it evolves by selecting for those who reproduce the most. But what is the exact mechanism of this evolution? Evolution occurs when small (or big) changes in genes occur by chance. If one of these changes helps the organism reproduce (by helping it live longer, catch more food, or tolerate a broader climate, for example) that change can be passed on to the organism's offspring.

Any change to a DNA sequence is called a *mutation*. Mutations occur naturally in DNA due to damage that is repaired improperly or errors in the copying of DNA. Mutations can be small, like switching one base for another, and create little or no change in the protein that the gene codes for. Or mutations can be large; whole sections of the genome can jump from one chromosome to another. In extreme cases, (which can result from a large or small change) the mutation creates a tangible change in the structure, function, or expression of a protein. These changes have more of a chance of altering behavior, survival, and reproduction of the whole organism, and thus can alter the evolution of that organism. The way in which changes in genes correlate to changes in the evolution of an organism is called *molecular evolution*.

Through an in depth analysis of the sequence and structure of genes, it is possible to tell how closely two organisms are related in evolutionary terms. By comparing the sequences of a given set of genes for two different organisms, we can determine how similar the genes are. Those species with very similar sequences are evolutionarily closer than those with very different gene sequences. Because small mutations tend to accumulate at a constant rate over time, these changes can also give us an idea of *when* new species evolved from common ancestors.

Thanks to molecular biology, the study of molecular evolution has progressed to the extent that it is possible to study the history of life on our planet, and evolution itself, at a molecular level.

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THE FUTURE OF MOLECULAR BIOLOGY

No one can predict what major advances in molecular biology will bring to mankind. But we can look at the field right now and guess where it is headed. Both genomics and proteomics are huge fields in molecular biology that are just beginning to be researched. *Genomics* is the field of science that aims to explain the function of every gene in our genome, as well as figure out how all of the DNA that doesn't code for genes works within the cell. *Proteomics* is a related field that hopes to describe how every protein functions, as well as how proteins interact with one another.

Stem cells are another tool that molecular biologists use, whose potential is nearly limitless. We spoke early of the ES cell, a totipotent stem cell derived from embryos. Scientists at Kyoto University have discovered how to make a powerful (pluripotent) stem cell from an adult skin cell (called an *induced pluripotent stem cell* or *iPS cell*). While this cell is a little more mature than an ES cell, it can still transform into any other cell or tissue!

Stem cell science is a very exciting field in molecular biology. Using these new technologies, scientists may be able to repair the damage in people with strokes, or those with kidney or heart failure. Induced pluripotent stem cells have the added advantage of being less morally questionable than embryonic stem cells, since they are made from adult tissue instead of embryos.

While molecular biology has the potential to drastically alter the way we treat diseases and live our lives, we cannot count on miraculous breakthroughs. Like any research science, progress in molecular biology is slow. However, all of the processes, techniques, and advances we have learned about come from the combination of creative thought with rigorous scientific methodologies.

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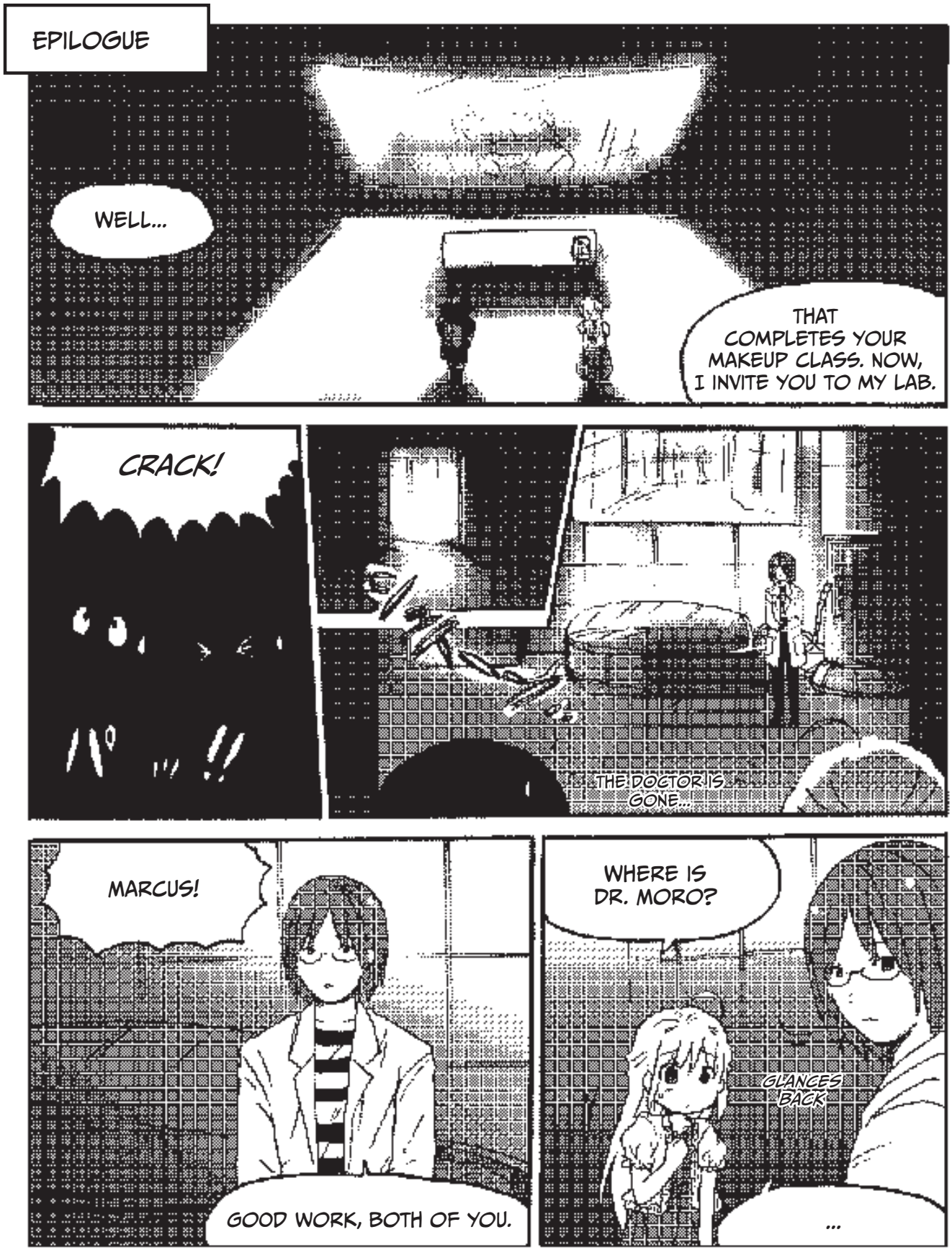

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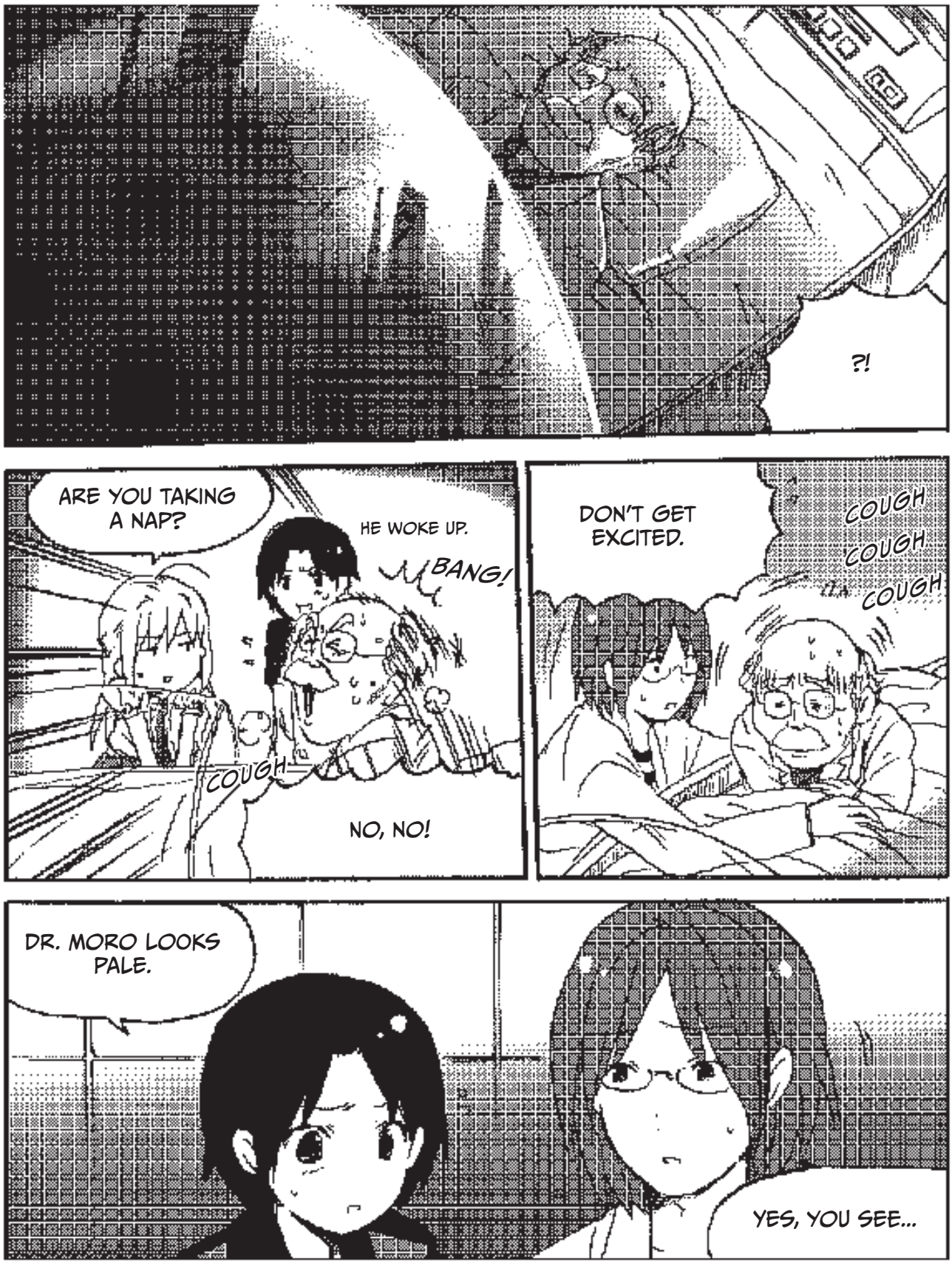

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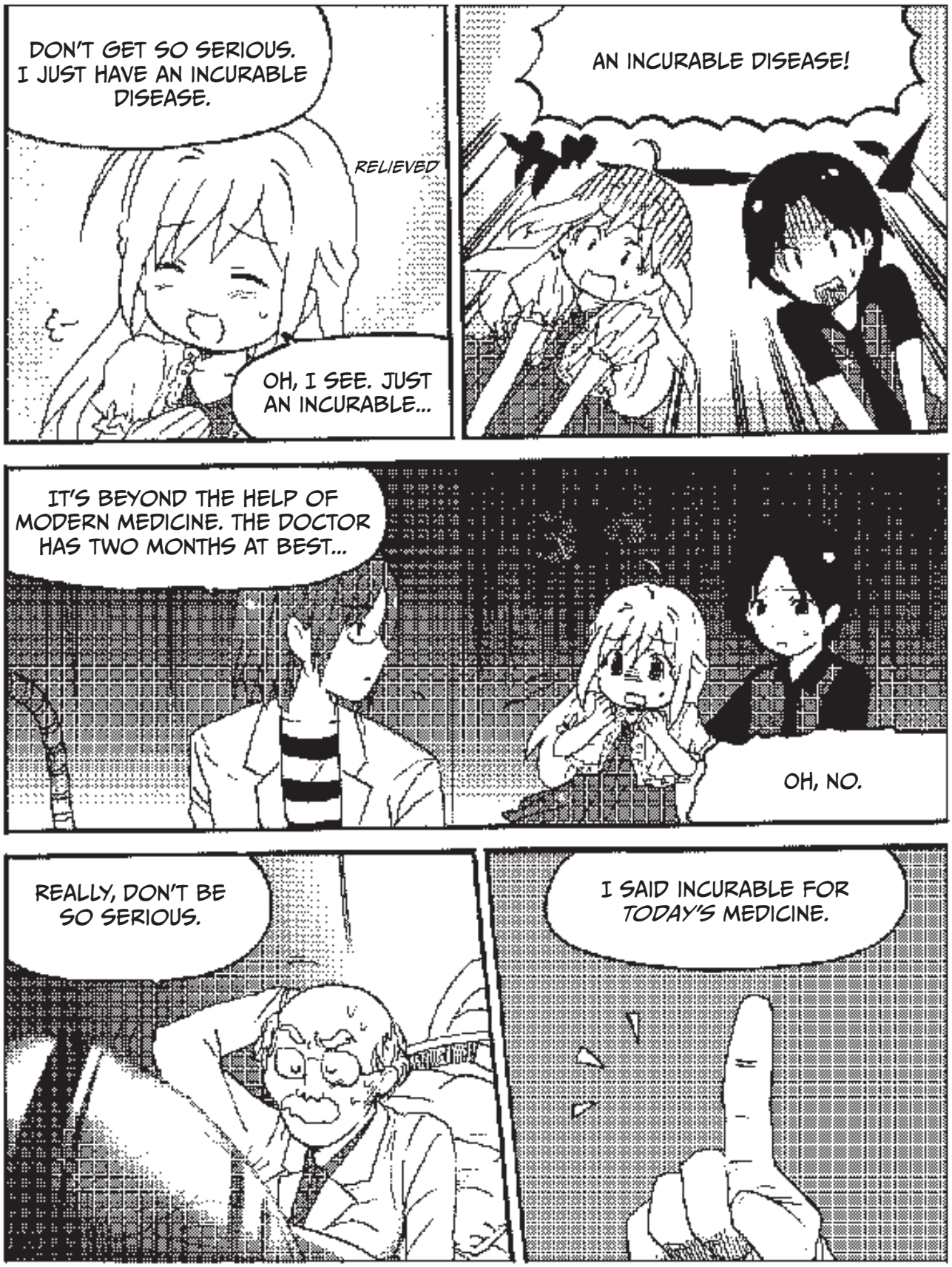

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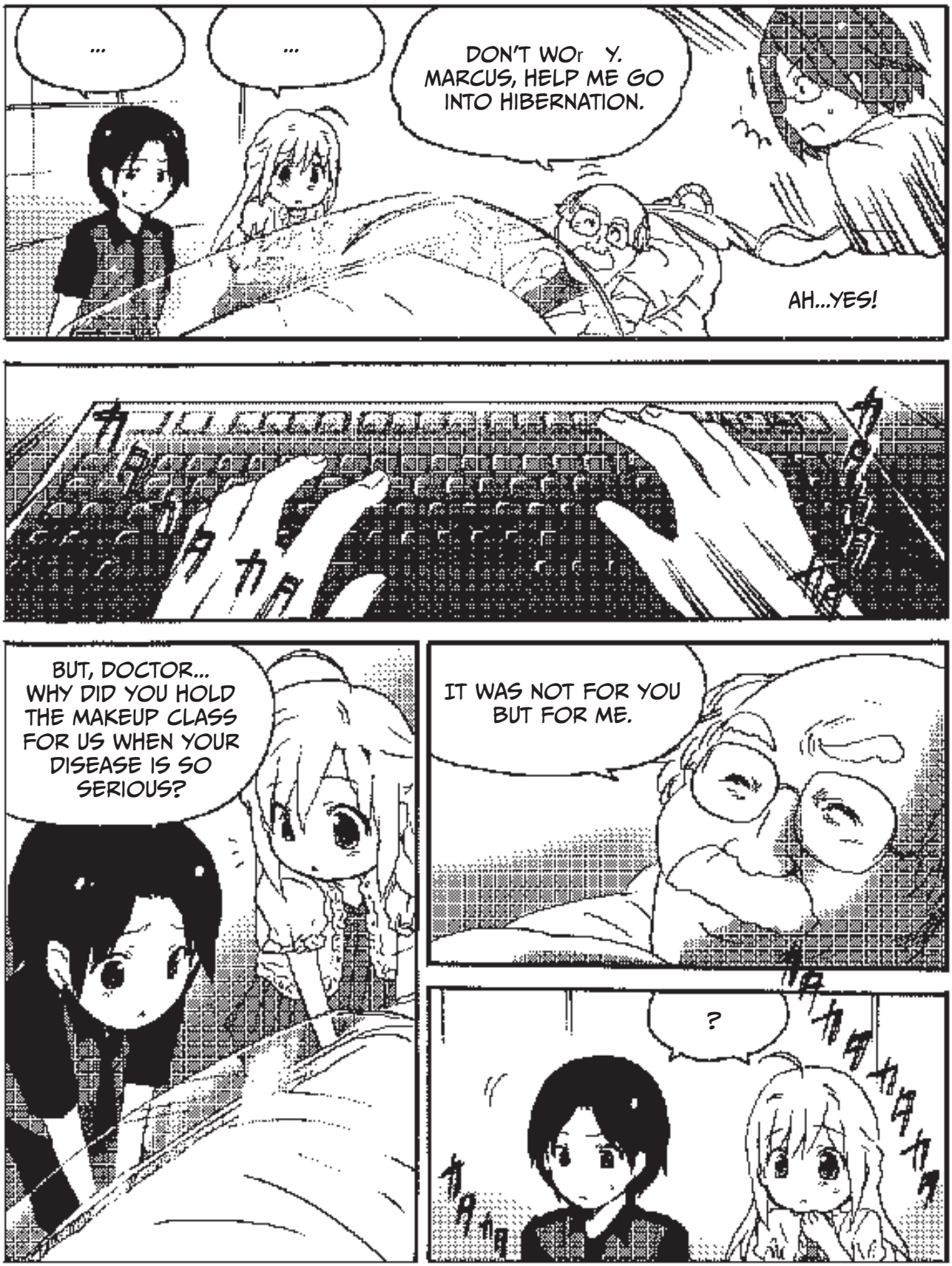

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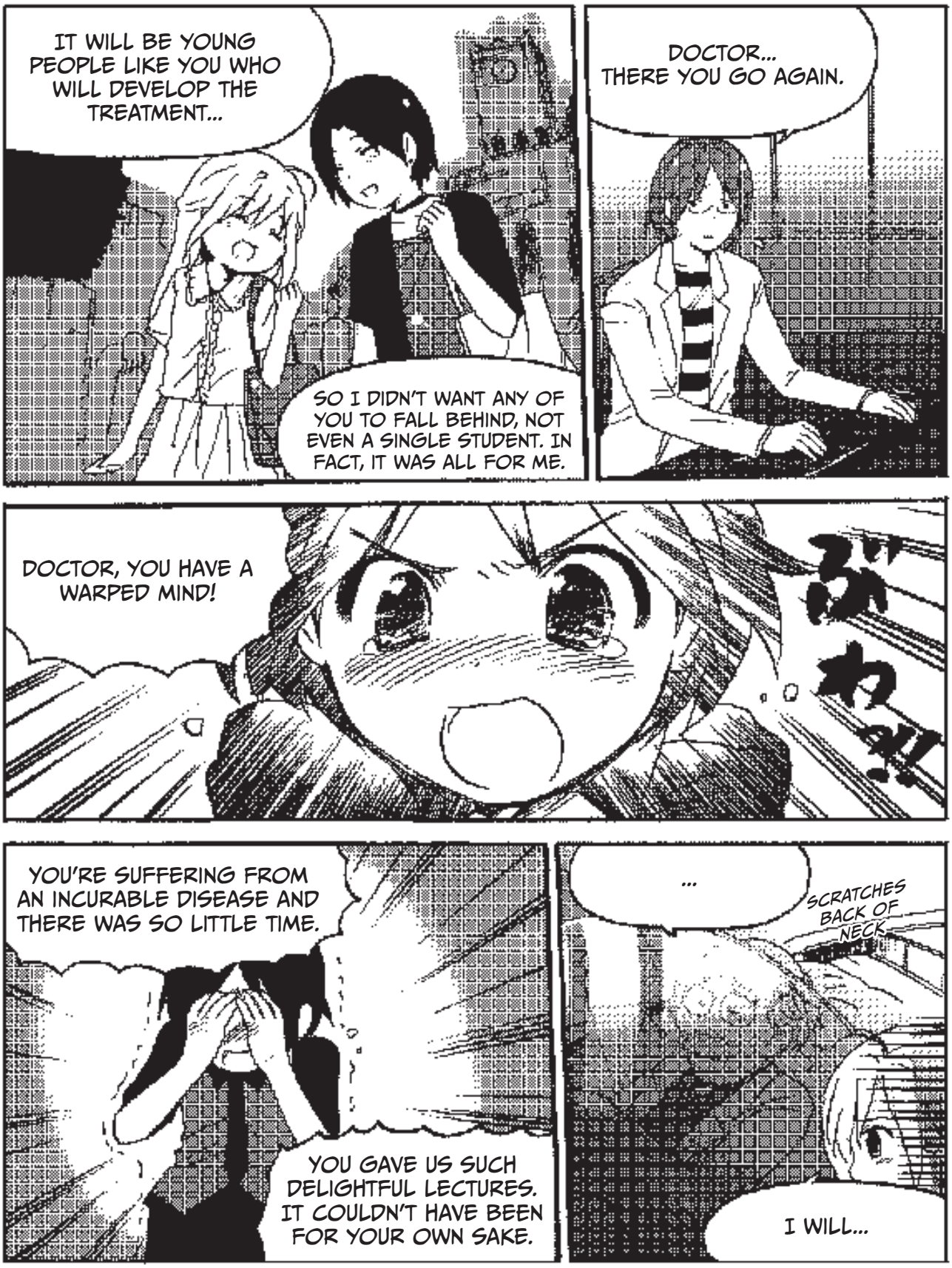

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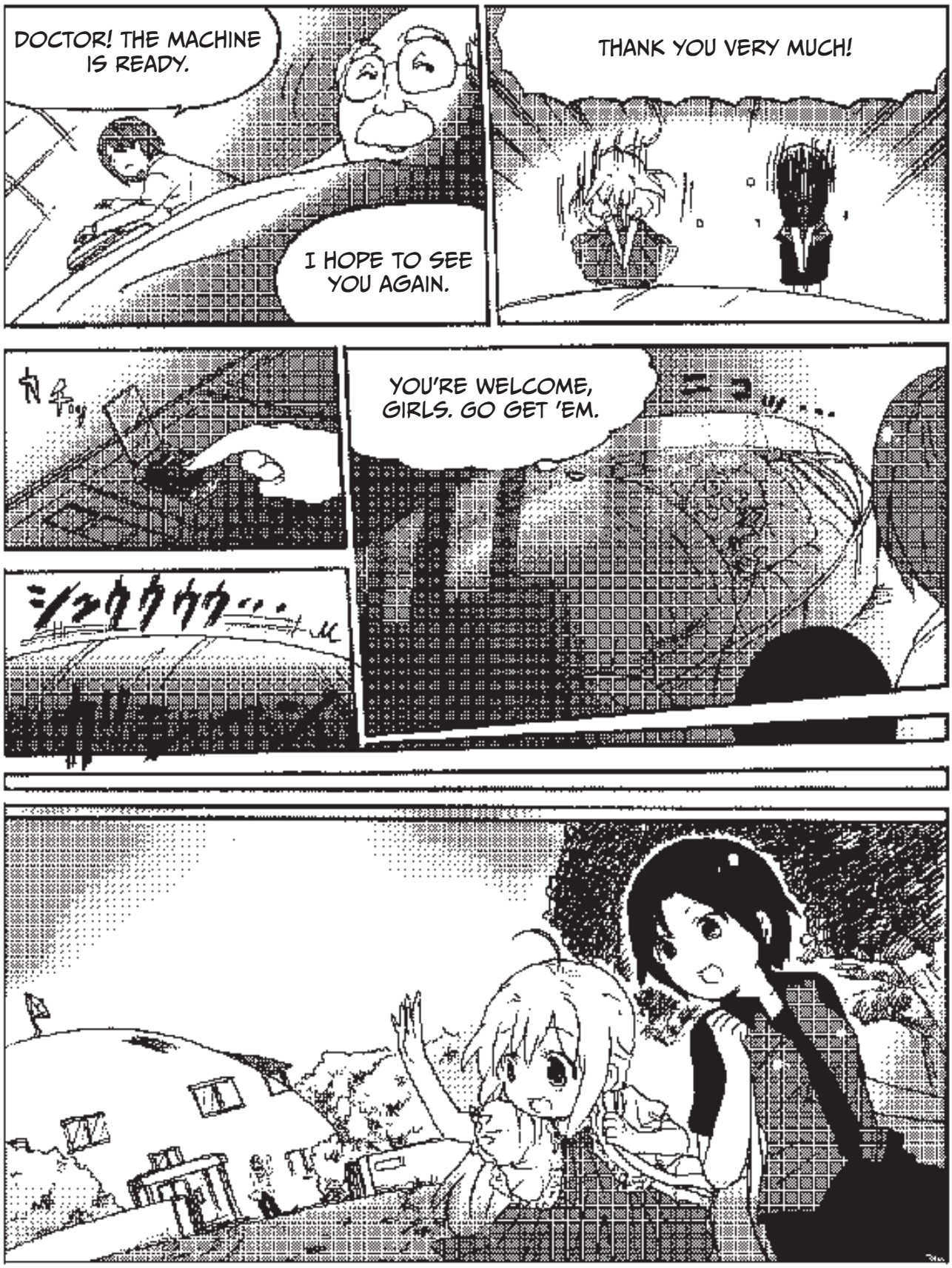

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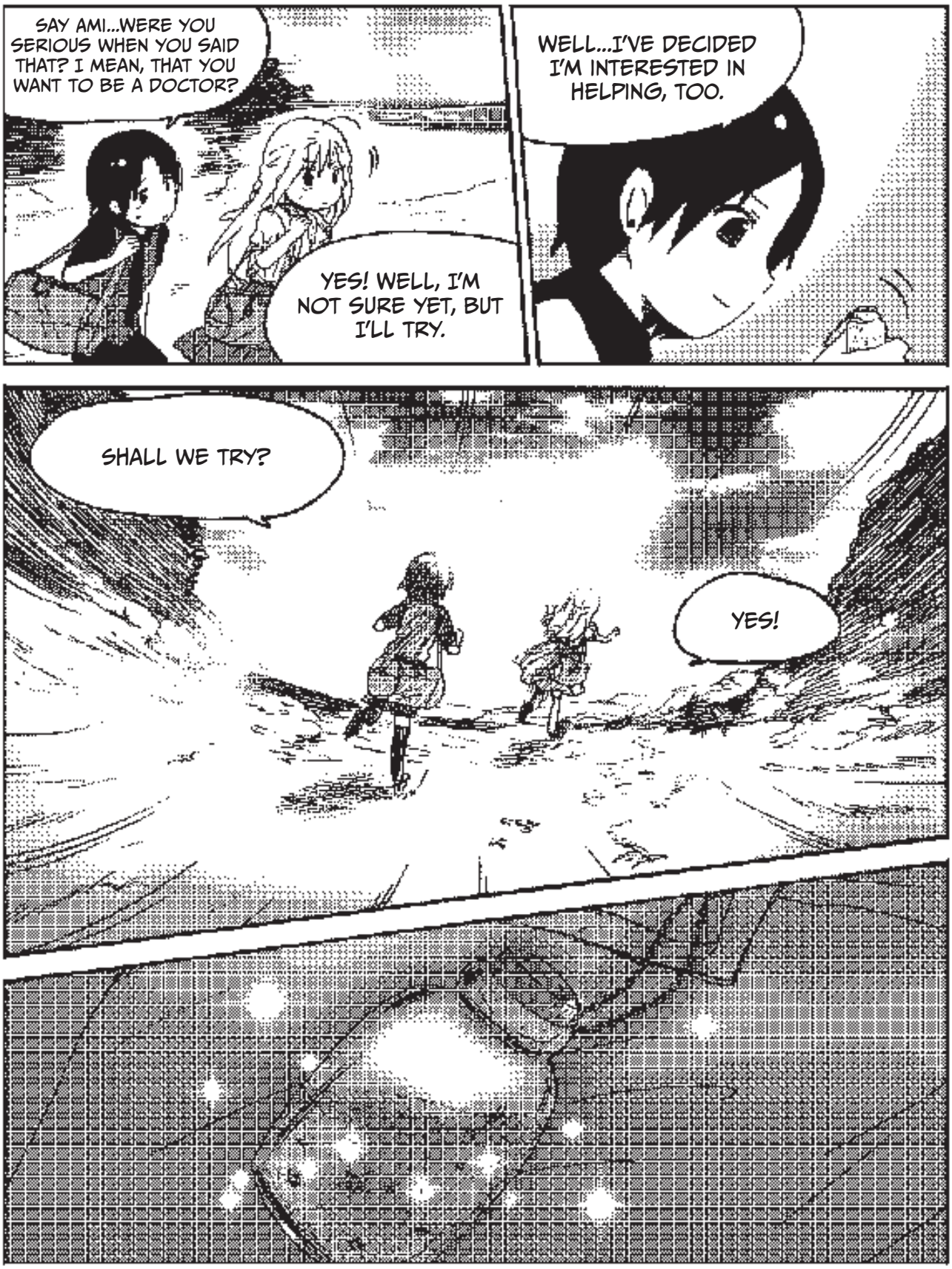

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ABOUT THE AUTHOR

Masaharu Takemura, PhD, has worked as a research associate at the Nagoya University School of Medicine (Japan), a visiting fellow at the University of Oxford (England), and a research associate at Mie University (Japan). He is currently an associate professor in the Faculty of Science at the Tokyo University of Science. He has published a number of research papers and books on biology-related subjects, including molecular biology, life science, and biology education.

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Production: Becom Co., Ltd.

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Tel: 03-3262-1161; Fax: 03-3262-1162

Scenario writer: Masayoshi Maeda

Illustrator: Sakura

Figure Creation: Smith Suzuki, Tsutada Design Planning

Cover Artist: Ogii Hagiwara

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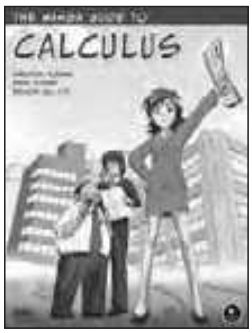

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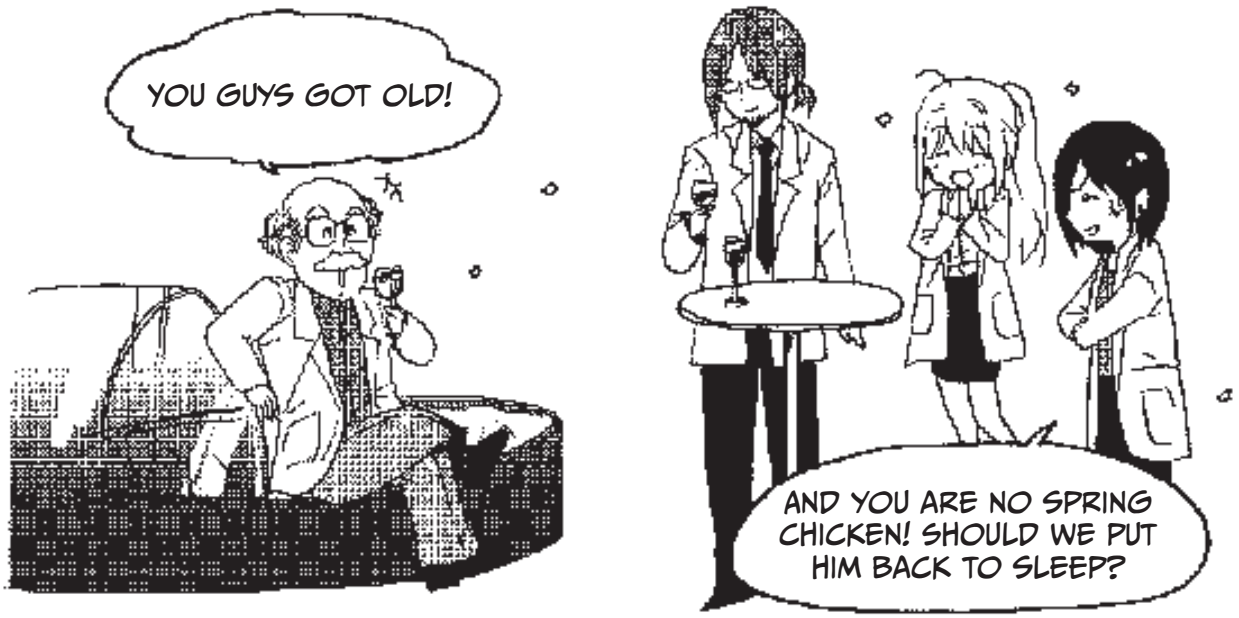

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